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### 8103 P*

## Comparison of Oral and Subcutaneous Administration of Protective Doses of Ascorbic Acid (Vitamin C)

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The administration of antiscorbutics other than the oral route was first tried by Hess and Unger.[1] They found that neutralized orange juice given subcutaneously produced no antiscorbutic effect, whereas given intravenously a marked curative effect was noted. Recently the intravenous administration of ascorbic acid has been employed clinically by several workers (Schultzer,[2] Kramár,[3] and Wright,[4]) with successful result, but the dosage used was apparently taken at random. Subcutaneous injection of ascorbic acid into guinea pigs was employed by Jadassohn and Schaaf[5] in connection with their study upon skin pigmentation. The dose used was very large, being 5-50 mg.

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* P represents a preliminary, C a complete manuscript.

[1] Hess, A. F., and Unger, L. T., PROC. SOC. EXP. BIOL. AND MED., 1918, **15**, 141.

[2] Schultzer, P., *Lancet*, 1933, **225**, 589.

[3] Kramár, E., *Deutsch. Med. Wochenschr.*, 1933, **59**, 1428.

[4] Wright, I. S., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 475.

[5] Jadassohn, W., and Schaaf, F., *Klin. Wochenschr.*, 1934, **13**, 845.

Using a sample of ascorbic acid obtained from the British Drug House aud employing Höjer's method of biological assay (diet same as that reported previously' Hou[6]) we found that with the same subminimal protective dose the degree of protection (graded according to Key and Elphick's[7] method) of 9 guinea pigs receiving the ascorbic acid by mouth was on the average exactly ½ that of 9 receiving the ascorbic acid by subcutaneous injection. According to Key and Elphick's interpretation, this result indicates that the minimal protective dose of ascorbic acid by oral administration is twice as large as that by subcutaneous injection. This may mean that part of the ascorbic acid given orally is possibly lost in the alimentary tract. Experiments are being carried out to test this point.

The ascorbic acid solution used was prepared fresh every other day and the remaining portion kept in a refrigerator. Before each feeding the actual content of ascorbic acid was determined by titration with iodine standard and also with 2-6 dichlor-phenol-indophenol. Oral administration was made by means of an accurate 1 cc. pipette and the subcutaneous injection by means of a 1 cc. tuberculin syringe, the solution being taken from the same bottle. Table I illustrates the result of one representative experiment. In each experiment 3 negative control animals showed 0 degree of protection, and 3 positive controls 4 degrees of protection.

TABLE I

Protection afforded by oral and subcutaneous administration of ascorbic acid (average daily dose 0.88 mg.)

| Oral Administration | | Subcutaneous Injection | |
|---|---|---|---|
| Guinea Pig No. | Degree of Protection | Guinea Pig No. | Degree of Protection |
| 386 | 1.5 | 390 | 3.0 |
| 385 | 1.0 | 388 | 2.0 |
| 389 | 1.5 | 387 | 3.0 |
| Aver. | 1.3 | Aver. | 2.7 |

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[6] Hou, H. C., *Chinese J. Physiol.*, 1935, in press.

[7] Key, K. M., and Elphick, G. K., *Biochem. J.*, 1931, **25**, 894.

## 8104 P

# Route of Migration of Spirocerca Sanguinolenta in Experimentally Infected Dogs.

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The Asiatic hedgehog, *Erinaceus dealbatus,* in the Peiping area is frequently infected with the third stage larvae of *Spirocerca sanguinolenta* encysted in omentum and mesentery as pointed out by Faust,[1] who infected experimentally dogs and cats by feeding them with these larvae and described the order of their migration in the animals as follows: Excystment in stomach cavity; penetrating stomach wall; passing into gastro-epiploic and portal veins; passing through hepatic capillaries, right heart, pulmonary capillaries and left heart; arriving in, and attaching themselves to the intima of, lower thoracic and upper abdominal aorta; migrating upward and finally settling in the wall of upper thoracic aorta. The part of the migration route within the aortic wall was described by Hiyeda and Faust.[2]

Being not completely convinced that the larvae can pass through 2 sets of capillaries whose calibers are much too small to provide an easy passage for them, and that they can penetrate the aortic wall in spite of the rapid blood flow in the aorta, we repeated Faust's feeding experiment and obtained the following results:

Twelve young dogs were each fed with 270 to 400 living encysted larvae. The dogs were killed by ether from 2 hours to 6 weeks after the feeding, and the organs, including the vessels of the stomach and the aorta are examined macro- and microscopically.

Our study shows that the larvae quickly excyst themselves in the stomach cavity and penetrate the stomach wall. After reaching the serosa they invade the arterial wall and migrate along the gastric and coeliac arteries, then the upper abdominal and the lower thoracic aorta and, finally, they reach the upper thoracic aorta which is their final habitat. The presence of larvae in the gastric and the arterial walls results in marked necrosis, acute inflammation, hemorrhage and formation of linear abscesses, all of which heal rapidly after the parasites have wandered on.

To determine whether the larvae can pass through the hepatic and pulmonary capillaries, 200 actively motile larvae, dissected out from the cysts and suspended in dogs' own blood, were injected respectively into one lung of one dog and the liver of another, everything being kept at body temperature to prevent the coiling up of the larvae. The blood coming out of these organs contained not a single larva, but in the lung and the liver mashed and digested for 3 to 4 days in 1% pepsin solution, 148 and 135 live larvae respectively were recovered.

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[1] Faust, E. C., PROC. SOC. EXP. BIOL. AND MED., 1927, **25**, 192.

[2] Hiyeda, K., and Faust, E. C., *Arch. Path.*, 1929, **7**, 253.

## 8105 P

# Treatment of Dirofilariasis Immitis with Concentrated Fouadin.

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*Dirofilaria immitis,* a viviparous filarial worm living in the right chambers of the heart, the pulmonary artery and its branches, is very prevalent in China, 7.7% of dogs in Canton and 27%* of dogs in Peiping being infested. No cure for the parasite was known until Itagaki *et al.*[1] and Wada[2] reported some success by using sodium antimony tartrate and neostibnal respectively. "Fouadin", a proprietary antimony compound was found to give highly satisfactory result by Philipp[3] and by Underwood and Wright,[4] who gave it a very thorough trial. Hays[5] also reported favorably on a double salt of antimony. Since the salts of antimony have given promising therapeutic result, the authors began in the winter of 1934 to test the efficacy of another complex antimony compound called "Concentrated Fouadin"† against this worm. A preliminary notice of the results is here given.

As subjects of experiment, naturally infected dogs were employed. To have an idea of the degree of infestation, the average of at least 3 consecutive daily counts of microfilariae in 0.025 cc. of dried and dehaemoglobinized blood obtained from the ear-vein at 4 p. m., was ascertained. For treatment, 16 dogs were divided into 2 groups. To Group I comprising 10 dogs, an intramuscular injection was given daily except Sundays until the microfilariae disappeared from the peripheral blood. The initial dose varied according to the size of the animal, generally 0.5 cc. for a dog under 10 kg. and 1 cc. for one weighing between 10 and 15 kg. For subsequent injections, the dose was gradually increased with the object of pushing the drug to the limit of tolerance as rapidly as possible. The 6 dogs of Group II were also injected intramuscularly, but after the first 2 consecutive daily doses corresponding to 0.05 cc. of Concentrated Fouadin per kg. body weight the third and the following injections were given on alternate days with a dose equal to 0.10 cc. per kg. body weight until the same result was achieved. As a 'follow-up' the same quantity of ear blood taken at 4 p. m. every 10 days was examined for the reappearance of microfilariae. When the dogs died or were sacrificed, searches for the adult worms were made in the heart and pulmonary arteries and in the nodules of infarcts in the lungs. Their viability was tested in warm normal saline. The uteri were cut open near the vulva and the condition of the escaping microfilariae and ova was noted. Microscopic sections were also made of any nodules found in the lungs.

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* From the records of Physiology Department, Peiping Union Medical College, Peiping, China.

[1] Itagaki, S., and Makino, R., *J. Japanese Soc. Vet. Sci.*, 1927, **6**, 1.

[2] Wada, K., *Scient. Rep. Gov't Inst. Infect. Dis., Tokyo Imp. Univ.*, 1927, **6**, 525.

[3] Philipp, F. G., *China J. Sci. and Arts,* 1931, **14**, 42.

[4] Underwood, P. C., and Wright, W. H., *J. Parasit.*, 1932, 180.

[5] Hays, J. M., *Vet. Med.*, April, 1933. Original not seen.

† Contains "11% Antimony III—pyrocatechin—disulphonate of sodium and calcium" supplied by 'Bayer'.

*Results.* Group I. Of the 10 dogs given daily injections: (a) 2 died of poisoning 2 days after receiving a total amount of 0.65 cc. and 0.38 cc. per kg. body weight of the drug spread over 4 and 5 days respectively. Autopsy findings: worms in right cardiac chamber and pulmonary artery still alive; those in hemorrhagic infarct nodules in the lungs (posterior lobes) dead. Other relevant data were: weight, 5.7 and 14.4 kg.; number of microfilariae per 0.025 cc. = 48 and 81. (b) The remaining 8, weighing from 6.7 to 15.4 kg. with microfilaria counts from 7 to 72 per 0.025 cc. were found free from microfilaria after 4- to 6-day courses of injections totally 2.5 cc. to 5.5 cc. They all appeared in a better condition than before treatment and gained weight, except one of the 2 dogs in which the larvae reappeared 11 days after treatment and which was not retreated. Three of them died from 3 weeks to 1 month after treatment as the result of surgical operation. They had received a total amount of 0.43 cc. and 0.50 cc. per kg. body weight. In 2 in which autopsy was done, the adult worms in the heart were still active. Two of the remaining 5 were killed, 78 and 86 days after the disappearance of microfilariae. Sixteen motile Dirofilariae were recovered from the hearts: 4 males and 6 females in the one and 2 males and 4 females in the other. The microfilaria counts before treatment were 24 and 12 per 0.025 cc. respectively. The total amount of Concentrated Fouadin used per kg. body weight was 0.26 cc. and 0.56 cc. The 3 survivors, except the one noted above, have been in good health and 141 days free from microfilariae up to the end of March, 1935.

Group II. Six dogs (weights from 8.8 to 16.4 kg.; microfilaria counts: 3 to 115 per 0.025 cc.) were treated on alternate days and the blood was rendered sterile from 11 to 14 days with a total dosage of 5.5 cc. to 10.10 cc. of Concentrated Fouadin. (a) Two were sacrificed 23 days and 110 days after treatment with total cc. per kg. body weight of 0.63 and 0.71 respectively and found to have firm infarcts in the periphery of the upper and lower lobes of both lungs; and one to have 5 pairs of living worms in the right auricles and the other, 15 males and 10 females in the same situation. The lung nodules contained dead worms. One female worm, 26 cm. in length, from each dog still had motile embryos *in utero*. (b) The remaining 4 dogs are still under observation and 3 of them have been negative for 136 days and the fourth for 170 days.

In one dog kept for control, the microfilariae have never disappeared from the circulation during the last 6 months.

Thus it is evident that by bringing the infected dogs under the toxic influence of Concentrated Fouadin as rapidly as possible through daily intramuscular injections, it was found possible to destroy the microfilariae but not all the adult worms which, however, except 2 out of 30, contained only dead embryos. By this method the microfilariae disappeared in from 4 to 6 days with a total of from 0.26 cc. to 0.69 cc. per kg. body weight. Whereas by the method of alternate day injections, it took from 11 to 14 days with the drug amounting to from 0.58 cc. to 0.71 cc. per kg. body weight (the last 2 doses in every case had to be given daily) to produce the same result. Compared with "Fouadin" from 21 cc. to 33 cc. of which, according to Underwood and Wright[4] were necessary to rid the dogs of the embryos, "Concentrated Fouadin" was 4 to 5 times therapeutically more potent. The largest single dose for dogs of from 12 to 15.5 kg. was 1.5 cc. as against 3 cc. for animals weighing 17.3 to 23.6 kg. with Fouadin. With the less amount of the drug required, the course (from 20 to 25 days for Fouadin) of treatment was also correspondingly as many times shorter.

## 8106 C

# Simple Technique for Studying Schistosome Worms in Vitro.

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The study of adult parasitic helminths is often handicapped by difficulties in keeping them alive for prolonged observation outside the body of their host. According to Coutelen[1] the first attempts at artificially rearing helminth parasites were made by Lönnberg in 1892 and Tower in 1900, using cestodes. Reichenow and Wülker[2] referred to the use of artificial media by Meggit in preserving the life of cestodes for several days and by Meier, who through frequent changes of media kept alive certain trematodes and cestodes from fish for as long as 21 days.

In metabolic studies of helminth parasites such as undertaken by Alt and Tischer[3] and Brand[4] observations must be made on living parasites *in vitro*. Such studies might have been more exhaustive if the life of the parasites could be maintained for several days instead of a few hours. In testing the anthelmintic value of a drug preliminary *in vitro* experiments seem an essential step and for such purposes intestinal helminths are usually used. Ryoji,[5] however, studied the action of certain organic antimony compounds on *Clonorchis sinensis in vitro* and Oesterlin and Krainick[6] tested the action of about 80 organic substances on many kinds of parasites including adult schistosomes. The latter authors remarked that the adult schistosomes recovered from experimentally infected mice do not remain alive in serum for long, probably as a result of bacterial growth in the medium.

In the course of chemotherapeutic studies in experimental schistosomiasis japonica the present authors devised a simple technique by which it was possible to keep adult schistosomes alive in artificial medium for weeks and if necessary for months without difficulty. This technique may prove useful in future studies in which it is desired to keep adult schistosomes or allied parasites alive for prolonged observation *in vitro*.

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[1] Coutelen, F., *Ann. de Parasit.*, 1927, **5**, 1.

[2] Reichenow, Ed., und Wülker, G., Leipzig, 1929, p. 125.

[3] Alt, H. L., and Tischer, O. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 222.

[4] Brand, Th., von., *Z. f. Vergleichende Physiol.*, 1933, **18**, 562.

[5] Ryoji, S., *Okayama Igakkai Zasshi*, 1927, **39**, 1809 (Abstract) *Trop. Dis. Bull.*, 1930, **27**, 959.

[6] Oesterlin, M., und Krainick, H., *Zentralb. f. Bakt., Parasit. u. Infekt.*, 1934, **132**, 222.

The difficulties in maintaining in artificial medium the life of such adult helminths as the schistosomes obtainable in an aseptic condition are mainly 2, accidental bacterial contamination and inspissation through evaporation of the medium. These difficulties can be surmounted by the use of a flask of the type employed in tissue culture work but smaller in size, which any competent glass blower can make to order (Fig. 1, A). The body of the flask is about 4 cm. in diameter and not more than 0.7 cm. in depth to permit observation under an ordinary low power objective (*e. g.*, Leitz No. 3) of the microscope without disturbing the contents. The mouth of the flask should be relatively wide, about 1.2 cm. in diameter, so as to allow easy introduction of the parasites and should be provided with a tight fitting rubber cap, which can be extemporized from the severed bulbous end of an ordinary rubber teat (Fig. 1, B, C). The flasks plugged with cotton wool and wrapped up in paper should be sterilized in the hot air sterilizer while the rubber caps placed in a petri dish are sterilized by steam under atmospheric pressure.

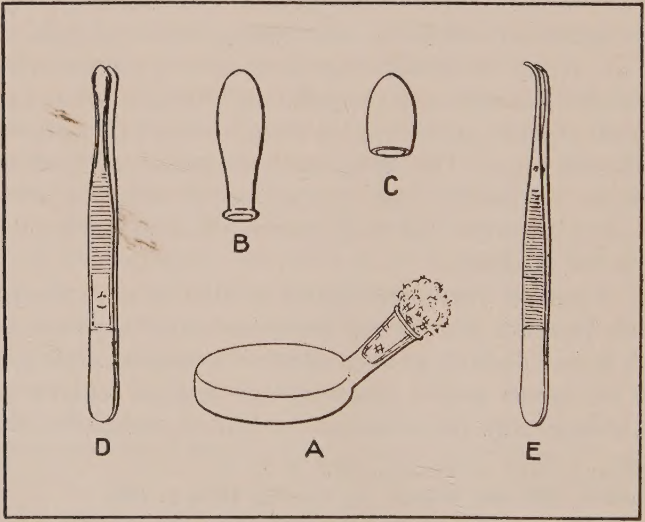

**FIG. 1.** For explanation of lettering see text.

The schistosomes are obtained from the large portal veins of an animal, preferably a rabbit on account of its large size, which has been infected 8 to 10 weeks previously with cercariae from snails. Rigid aseptic technique must be practiced in laying open the main portal veins entering the hilum of the liver. In extracting the worms from the lumen of the veins a pair of forceps with round and smooth tips (Fig. 1, D) should be used to avoid injury to the parasites. They are then first transferred to a small covered petri dish containing warm sterile Locke's or saline solution and later distributed in a number of culture flasks into each of which exactly 2 cc. of the medium to be used have been previously introduced by means of a 10 cc. pipette under bacteriological technique. In carrying the worms over the neck of the flask a pair of fine, curved forceps (Fig. 1, E) are of advantage. The culture flasks with their mouths covered by rubber caps are then placed in an incubator at 37°C. to simulate the natural environment of the schistosomes.

Daily or, when indicated, hourly observations should be made of the culture to ascertain the condition of the worms. Any culture, in which there is evidence of bacterial contamination by a cloudy appearance of the medium should be discarded. The condition of the worms may be judged by their movements. Those showing no movements can be induced to do so by gently heating the flask over an electric hot plate unless they are actually dead. No worm should be regarded as dead without passing this test. Worms becoming sluggish after a few days will show renewed activity upon a change of fresh medium.

Almost any animal blood sera will serve as satisfactory media. We have tried horse, sheep and rabbit serum and ascitic fluid from cases of cirrhosis of liver with success. The addition of Locke's solution or dextrose does not improve the efficiency of the media. The duration of life of the worms in different media varies somewhat depending probably more upon the inherent vitality of the parasites rather than upon the nature of the medium.

Table I shows the results of a typical experiment. In this experiment it would seem that ascitic fluid and sheep's serum are more efficient than horse or rabbit serum but in another experiment some worms lived for 21 days in rabbit serum. With changes of fresh

TABLE I.

Duration of Life in Days of Schistosomes in Different Media without Change.

| | Horse serum | Sheep serum | Rabbit serum | Ascitic fluid |
|---|---|---|---|---|
| Copulating pairs—Males | (2) 13-14 | (2) 18 | — | (2) 13-14 |
| Females | (2) 9-11 | (2) 14-15 | — | (2) 19-20 |
| Mature males | (6) 9-22 | (7) 13-19 | (9) 7-10 | (4) 14-16 |
| Mature or almost mature females | (2) 8-17 | (3) 8-9 | — | — |
| Immature females | (5) 8-16 | (5) 10-14 | (8) 7-9 | (5) 17-20 |
| Aver. | 13.4 | 14.4 | 8.2 | 16.3 |

Figures in parentheses indicate number of worms studied.

medium at intervals it is possible to prolong the life of the schistosomes for 2-3 months. In one experiment in which the medium (in this case rabbit serum) was renewed at intervals of 1-2 weeks 2 schistosomes lived for 82 days.

It was also found that not more than 4 or 5 worms should be placed in each flask. Overcrowding shortens the duration of life of the parasites as shown by the experiment recorded in Table II.

TABLE II.
The Effects of Overcrowding. The Worms Were All Mature Males Uniform in Size. Plain Ascitic Fluid Was the Medium Used. No Change of Medium.

| No. of flasks | No. of worms in each flask | Aver. duration of life in days |
|---|---|---|
| 3 | 3 | 16 |
| 3 | 6 | 13 |
| 3 | 9 | 11 |
| 3 | 12 | 10 |
| 1 | 30 | 8 |

*Summary.* A simple technique is described for maintaining the life of adult schistosomes *in vitro* over a period of several weeks which, with frequent changes of medium, may be extended to 2½ months. This is made possible by the use of small tissue culture flasks, which prevent bacterial contamination and desiccation of the media. The latter may be either horse, sheep or rabbit sera or human ascitic fluid.

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### 8107 C

## Action of Various Organic Antimony Compounds on Schistosoma Japonicum in Vitro.

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By using the technique described by Lee and Chu[1] studies were made of the lethal action of the following 4 antimony compounds on adult *Schistosoma japonicum in vitro.*

### *Trivalent Antimony Compounds*

Sodium antimonyl tartrate; chemically pure, supplied by E. Merck, Germany.

Fouadin. This is a 6.3% solution of a colorless powder, antimony III-pyrocatechin-disulphonate of sodium, supplied by Bayer and Company, Leverkusen, Germany.

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[1] Lee, C. U., and Chu, H. J., in press.

### *Pentavalent Antimony Compounds*

Urea-stibamine. A slightly pinkish chalky powder, the principal constituents of which are, according to Gray and Trevan,[2] a substance corresponding in composition to the di-substituted urea, symdiphenyl-carbamide-4:4′-distibinic acid, and antimonic acid; supplied by Brahmachari Research Institute of Calcutta, India.

Neostibosan. A yellow powder, p-amino-phenyl stibinate of diethylamine, supplied by Bayer and Company, Leverkusen, Germany.

In all experiments an exact amount of 0.05 cc. of the drug to be tested in various dilutions was introduced by means of a tuberculin syringe into each flask containing 3 or 4 schistosomes in 2 cc. of medium. For purposes of comparison with fouadin, which comes from the manufacturers in a 6.3% solution of the powdered drug, urea-stibamine and neostibosan were also made up in 6.3% solutions from which dilutions were made.

The results of the experiments are presented in Tables I and II.

TABLE I.
Lethal Action of Sodium Antimonyl Tartrate on *Schistosoma Japonicum in Vitro.*

| Dilution | Mg. of sod. ant. tart. in 2 cc. | Sheep serum | Ascitic fluid |
|---|---|---|---|
| 1 to 10,000 | 0.2 | 30 min. | 30 min. |
| 20,000 | 0.1 | 35 ” | 34 ” |
| 40,000 | 0.05 | 1 hr. | 59 ” |
| 80,000 | 0.025 | 5 ” 45 min. | 1 hr. 25 ” |
| 100,000 | 0.02 | 10 ” 30 ” | 1 ” 35 ” |
| 200,000 | 0.01 | — | 19 ” 8 ” |
| 400,000 | 0.005 | — | 21 ” 8 ” |
| 800,000 | 0.0025 | — | 44 ” 45 ” |
| 1,600,000 | 0.0013 | — | 4½ days |
| Control | | 13 days | 15 ” |

The results of these experiments show clearly that the trivalent antimony compounds are more potent in their lethal action on the schistosomes than the pentavalent compounds and that sodium antimonyl tartrate is the most and neostibosan the least potent of all. This agrees with the prevalent belief that trivalent antimony compounds are more effective in the treatment of schistosomiasis than the pentavalent salts and with our own clinical experience that permanent cure of *Schistosomiasis japonica* results more readily with tartar emetic than with fouadin.

If the average weight of a Chinese patient suffering from schistosomiasis is taken to be 50 kg., the weight of his blood will be 3846 gm., a thirteenth part of his body weight, and the actual vol-

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[2] Gray, W. H., and Trevan, J. W., *J. Roy. Soc. Trop. Med. and Hyg.*, 1931, **25**, 147.

## TABLE II.

Lethal Action of Fouadin, Urea-stibamine, and Neostibosan on *Schistosoma Japonicum in Vitro.*

| Dilution of 6.3% solution of antimony compound | Mg. of antimony compound in 2 cc. | Fouadin | | Urea-stibamine | | Neo-stibosan | |
|---|---|---|---|---|---|---|---|
| | | Sheep serum | Ascitic fluid | Sheep serum | Ascitic fluid | Sheep serum | Ascitic fluid |
| 1 to 400 | 0.315 | 3 hr. | 2 hr. 50 min. | 15 hr. | 3 hr. 15 min. | 8 days | 5½ days |
| 600 | 0.210 | 3 " 37 min. | 4 " 12 " | 28½ " | 15 " 10 " | 8 " | 19 " |
| 800 | 0.157 | 8 " 21 " | 4 " 26 " | 31 " | 16 " 20 " | 4 " | Culture infected |
| 1,000 | 0.126 | 9 " | 4 " 30 " | 31 " | 17 " 10 " | 8 " | 4 days |
| 2,000 | 0.063 | 12 " 36 min. | 5 " 30 " | 54 " | 42 " | 9 " | 20 " |
| 4,000 | 0.032 | 25 " 50 " | — | — | — | — | — |
| 8,000 | 0.016 | 25 " | — | — | — | — | — |
| 16,000 | 0.008 | 56 " 15 min. | — | — | — | — | — |
| 32,000 | 0.004 | 67 " | — | — | — | — | — |
| 64,000 | 0.002 | 111 " | — | — | — | — | — |
| Control | | 13 days | 27 days | 13 days | | 17 days | |

ume, 3663 cc., the specific gravity of blood being 1.050. In introducing intravenously 0.1 gm. of sodium antimonyl tartrate, a safe largest single dose, one obtains a concentration of one in 36,630. Since the cellular elements of the blood occupy at least one-third of the blood volume and there is no reason to believe that the blood cells take up any of the drug, the concentration of sodium antimonyl tartrate in the plasma is actually much higher than 1 in 36,630. *In vitro* even such a concentration of sodium antimonyl tartrate would kill the schistosomes in less than one hour. This, however, does not necessarily argue a direct action *in vivo* as it is well known that antimony compounds introduced intravenously leave the blood stream in a matter of a few minutes.[3] Thus one may not draw any conclusion from these *in vitro* experiments as to the nature of the anti-schistosomal action of antimony compounds *in vivo*.

*Summary.* The lethal action of sodium antimonyl Tartrate, Fouadin, Urea-stibamine and Neostibosan on *Schistosoma japonicum* is studied *in vitro* and the results support the prevalent belief that the trivalent antimony compounds are more effective in the treatment of schistosomiasis than pentavalent salts and our own clinical experience that permanent cure of *Schistosomiasis japonica* results more readily with tartar emetic than with fouadin.

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## 8108 C

## Liquefication of Rabbit Fibrin-Clots by Concentrated Streptococcus Fibrinolysin.

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From the observations of Tillett and Garner[1] and Madison,[2] the fibrinolysin from human strains of *Streptococcus hemolyticus* seems to act specifically on human plasma or fibrin-clots. Although Tillett and Garner have noted exceptional instances of slow dissolution of rabbit fibrin clots by culture of *Streptococcus hemolyticus*, the homologous rabbit plasma or fibrin clots are generally found to be resistant to the fibrinolysin while clots composed of human and rabbit heterologous fibrinogen-thrombin complexes are readily liquefied by it. The mechanism responsible for the resistance of the homologous rabbit fibrin-clots to action of the fibrinolysin is still unexplained. In an attempt to throw some light on this point, the present experiment was carried out with the use of concentrated streptococcus fibrinolysin.

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[3] Willaud, H., and Behrens, B., *Handbuch der Experimentellen Pharmakologie*, Berlin, 1927, **3**, Part I, 533.

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[2] Madison, R. R., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 641.

Six strains of *Streptococcus hemolyticus,* isolated from sore throat and scarlet fever cases, were used for production of fibrinolysin. Sixteen hours' growth of the culture in meat-infusion broth (initial pH 7.6) containing 1% dextrose was passed through a Seitz filter. The filtrate was concentrated according to the alcohol precipitation technique of Garner and Tillett.[3] The precipitate after first alcohol precipitation was washed once with a fresh lot of cold 95% alcohol and recollected by centrifugation. The recollected precipitate was first dried by a vacuum suction pump and then redissolved in saline to a volume equal to 1/40-1/20 that of the original culture filtrate. The insoluble particles in the solution thus obtained were removed either by filtration through filter paper or by centrifugation. The clear filtrate or supernatant fluid was a highly concentrated fibrinolysin solution and was used for tests in the present experiment. Fibrinogen and thrombin from plasma of man, rabbit and guinea pigs were prepared according to the method used by Tillett and Garner. In order to facilitate performing many tests with a small amount of materials, the original technique of the fibrinolytic test of Tillett and Garner was modified as follows: To 0.2 cc. of fibrinogen diluted 1:5 with saline, 0.2 cc. of the concentrated fibrinolysin and 0.1 cc. of thrombin solution were added and thoroughly mixed. The mixture is allowed to stand in incubator at 37°C. In these tests human and rabbit fibrin-clots promptly formed within 1-2 minutes and those of guinea pig from 5 to 10 minutes. With the concentrated fibrinolysin preparations, the homologous human fibrin clots were completely liquefied in 3-5 minutes and those of rabbit in 30 to 180 minutes, while the homologous fibrin clots of guinea pigs were not liquefied even after 24 hours' incubation at 37°C. Although there is a considerable degree of variation in the liquefaction activity of different lots of fibrinolysin prepared from different strains, the results clearly show that rabbit fibrin clot is not resistant to the streptococcus fibrinolysin if the fibrinolysin employed is of sufficiently high concentration.

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[3] Garner, R. L., and Tillett, W. S., *J. Exp. Med.*, 1934, **60**, 239.

### 8109 C

## Variation in Streptococcus Fibrinolytic Action on Human Plasma.

H. W. YÜ AND SAMUEL H. ZIA. (Introduced by T. J. Kurotchkin.)

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Tillett and Garner[1] have shown that filtrates of young broth cultures of hemolytic streptococci of human origin can liquefy fibrin clots of normal human plasma. Tillett, Edwards and Garner[2] further found that resistance to this fibrinolytic action in plasma of patients during convalescence from streptococcus infections might indicate antibacterial immunity to that organism. Myers, Keefer and Holmes[3] recorded that this resistance in plasma of patients with rheumatic fever is comparable to that observed in plasma of patients with hemolytic streptococcus infections. Both groups of investigators used the same strain of organism having high fibrinolytic action (*i. e.*, strain CO) as the test organism. Hadfield, Magee and Perry,[4] however, called special attention to the variation in the fibrinolytic activity of different strains of hemolytic streptococci and they proposed to use at least 3 strains of lytic organisms in all tests. In view of the importance of this test in immunological studies, it occurred to us to determine the extent of the variation in the fibrinolytic action of different streptococci of human origin on plasma from normal individuals as well as from patients suffering from acute hemolytic streptococcus infections. An attempt to find a possible relation between this difference of action and the organisms isolated from different groups of diseases due to streptococci was also made at the same time.

The original technique of Tillett and Garner[1] was followed. All except one strain of streptococci were recovered from patients suffering from acute infections, the other being Dochez N. Y. 5, which was obtained from stock. They were grown in 0.05% dextrose meat infusion broth, pH 7.6, for from 18 to 24 hours before use. Blood specimens were collected by venapuncture into tubes containing 0.01 gm. potassium oxalate per cc. of blood. The plasma, after being separated by centrifugation, was tested within

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[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[2] Tillett, W. S., Edwards, L. B., and Garner, R. L., *J. Clin. Invest.*, 1934, **13**, 47.

[3] Myers, W. K., Keefer, C. S., and Holmes, W. J., *J. Clin. Invest.*, 1935, **14**, 119.

[4] Hadfield, D., Magee, V., and Perry, C. B., *Lancet*, 1934, **1**, 834.

24 hours. The actual test was assembled by mixing 0.2 cc. plasma diluted with saline to one cc., 0.5 cc. broth culture, and 0.25 cc. of 0.25% calcium chloride in normal saline. The mixture was incubated at 37°C. in a water bath. The time interval between clotting and complete dissolution of the clot was recorded in hours or fractions thereof. Any clot not dissolved in 24 hours was considered to be resistant.

A preliminary study of 19 strains of streptococci on a few specimens of normal human plasma confirmed the observation that under uniform conditions, different strains of hemolytic streptococci showed marked difference in ability to produce fibrinolysin.

From the data obtained, the following deductions might be made. The variation in the fibrinolytic power of different strains of hemolytic streptococci for normal human plasma is not uniform. While a rough correlation for each strain undoubtedly exists, the fibrinolytic action of each strain of streptococcus may vary as much as from 6 to over 24 hours. Even with organisms of fairly equal activity, the susceptibility of plasma of patients with streptococcus infections is again markedly different. In 2 instances, this difference was 5 hours, while in 4 other cases, it was more than 24 hours. Moreover, this difference in susceptibility of the plasma did not correspond to the different sources from which the strains were obtained. Plasma from a patient convalescing from scarlet fever has been shown to be resistant to a streptococcus isolated from a patient with puerperal sepsis, but susceptible to some of the scarlet fever strains. In view of the existence of so much variation in streptococcus fibrinolytic activity even among such a small series, caution in interpreting the results of this test as an index of immunity to streptococcus infections is indicated.

## 8110 P

## Protection Against Experimental Typhus Infections (Peiping Strain) with Immune Mexican Typhus Serum.

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Zinsser and Castaneda[1] were able to produce an effective anti-typhus serum by prolonged immunization of a horse with formalinized suspensions of Mexican typhus Rickettsiae. With it they could protect guinea pigs not only against infections of Mexican typhus, but also completely against that of the European type.[2] In our study of typhus fever in North China, we have had an opportunity to examine whether or not this serum would have any definite protective action against the local strain. A brief preliminary report on this study follows:

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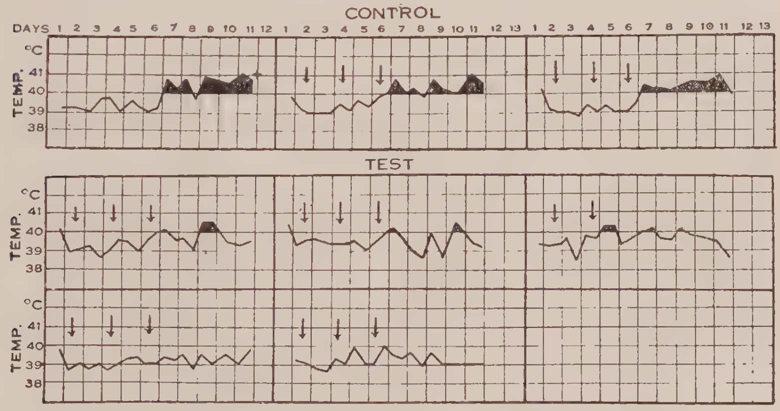
[1] Zinsser, H., and Ruiz Castaneda, M., *J. Exp. Med.*, 1933, **57**, 391.

The typhus strain used was obtained from a patient with typhus fever in Peiping. Gajdos and Chang have used it for the preparation of vaccine by the Weigl's louse intestine method in the last few years.[3] The immune horse serum was prepared in the Massachusetts State Serum Laboratory under the supervision of Zinsser and Castaneda. Male guinea pigs weighing between 300 and 400 gm. were used as experimental animals. One-fifteenth of a saline suspension of the brain of an infected guinea pig was injected intraperitoneally into a number of guinea pigs, and 24, 72, and 120 hours later, 1 cc. of immune or normal horse serum was injected subcutaneously. A control animal which received no serum was included. The body temperature was taken twice daily. The record of only one experiment is presented in the accompanying charts. The result of the other experiment was essentially the same.

The practical usefulness of the important finding of Zinsser and Castaneda is such that it needs no special comment. They offered



These charts represent the records of body temperature of 3 control and 5 test guinea pigs. Those readings that are elevated above normal are indicated by the shaded areas. The arrows show the points at which serum, normal or immune, was injected.

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[2] Zinsser, H., and Ruiz Castaneda, M., *J. Exp. Med.*, 1934, **59**, 471.

[3] Gajdos, S., and Chang, J., *Chinese Med. J.*, 1933, **47**, 441.

a simple and possibly a successful method to treat and control this serious epidemic disease. Before applying this method to clinical trial here in China, we have endeavored to study its effectiveness in experimental infections. So far, the results obtained seem to indicate that with the dose employed, a significant protection has been obtained by means of this immune horse serum. The exact dosage and the possible therapeutic use of the serum after the appearance of the fever are subjects now under study.

We wish to acknowledge our indebtedness to Professor Zinsser of Harvard Medical School, Boston, for a liberal supply of immune serum, and to Dr. J. Chang of the Catholic University, Peiping, for the typhus strain used.

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## 8111 P

## Effects of Cobra Venom on the Fujinami Rat Sarcoma.

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*From the Departments of Bacteriology and Immunology and Surgery, Peiping Union Medical College, Peiping.*

Calmette[1] has reported that the injection of cobra venom into a mouse carcinoma brought about a very rapid disappearance of the tumor, and he suggested that cobra venom might be of value in the treatment of other tumors. Monaelesser and Taguet[2] injected cobra venom into the carcinomas of man, and they reported encouraging results.

Our experiments were performed for the purpose of determining the effect of cobra venom when injected into the Fujinami rat sarcoma. The minimum lethal dose of cobra venom for white rats, weighing from 150 to 250 gm. each, was found to be one cc. of a 1:10,000 dilution. The solutions of cobra venom used were prepared according to the following technic described by Calmette. A 10% solution of desiccated cobra venom in normal saline was prepared and heated at 72°C. for 30 minutes. The solution, including the precipitate which was formed, was then centrifuged, and the clear portion was placed in ampoules and kept as a stock solution.

Series 1. Five rats were inoculated with bits of the Fujinami rat sarcoma which reached an average size of 0.5 x 0.5 x 1 cm. in

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[1] Calmette, A., *C. R. de l'acad. de Sc.*, 1933, **197**, 205.

[2] Monaelesser et Taguet, C., *Bul. de l'acad. de Med.*, 1933, **109**, 371.

10 days. The injections, which were always made in different parts of the tumor, were then begun, the first of which consisted of 1 cc. of a 1:100,000 dilution of cobra venom. Subsequent injections of 1 cc. were given every other day, but the concentration of cobra venom was gradually increased until at the fourth injection a concentration of 1:25,000 was reached. By the time that the seventh injection had been given the tumors were from 3 to 5 times their size at the time of the first injection, and the skin was beginning to ulcerate. Each animal was then killed. At autopsy, without exception, a large part of each tumor was necrotic. However, there remained a considerable amount of tumor tissue which appeared uninjured.

Series 2. Twelve rats, varying in weight from 100 to 290 gm., were inoculated with bits of Fujinami rat sarcoma. Two weeks later the tumors of 10 of these rats were injected with cobra venom, the doses being from 1 cc. of 1:100,000 to 1:50,000 dilution given every other day. An attempt was made to inject only the peripheral portions of the tumors. The remaining 2 rats had their tumors injected in a similar manner with 1 cc. of physiological saline solution instead of with cobra venom. The 10 experimental animals died after having received from 4 to 8 injections of the venom. The 2 control animals were then killed. The autopsy performed in each instance revealed that there was extensive necrosis in the tumors which had received cobra venom while those in which the normal saline had been injected were free from any damage. However, the size of the tumors in the experimental and in the control animals was the same.

Series 3. Twelve rats, weighing an average of 300 gm. and which had been given cultures of Sporotrichum for over a year in connection with another experiment, were inoculated with the Fujinami rat sarcoma in the usual manner. At the end of 24 hours, and every 48 hours thereafter, injections of cobra venom were made into or around the tumor. During the first 5 days, it was difficult to determine the exact spot where the tumor transplant was, but it then became palpable in every animal.

Four rats were treated with physiological saline solution, and 8 were given cobra venom, beginning with a 1:100,000 dilution and ending with 1:12,000 dilution. Four rats died after receiving only 2 injections of cobra venom, and at autopsy only a small area of necrosis was found in the tumors. Two other animals died after 7 injections of cobra venom. The remaining 2 rats were given no more venom but were kept 36 days longer together with the 4 control rats.

At the end of this time the tumor in each animal occupied the whole abdominal area. There was no ulceration or discharge. The 6 animals were killed and at autopsy no metastases were found. The tumor in each control animal was highly vascular and no necrotic areas were observed. In each experimental rat the tumor consisted of a thin layer of active sarcomatous tissue which surrounded an extensive central area containing a large amount of hemorrhagic fluid.

*Conclusions.* These data indicate that cobra venom is a destructive agent for the cells of the Fujinami rat sarcoma, but its therapeutic value is limited because of its localized action on the tumor cells, leaving the unaffected cells free to grow at a rapid rate.

### 8112 P

## Effect of Intracisternal Injections of Acetylcholine.

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Dogs under ether anesthesia were used for all the experiments. Blood pressure and respiration were recorded. Intravenous injections of acetylcholine were given through a cannula in the femoral vein and the intracisternal injections were given through a needle previously introduced into the cisterna magna. The needle was kept in place throughout the experiment.

In a series of 11 dogs intracisternal administration of acetylcholine gave an elevation of blood pressure in all the animals. In some of them an exaggeration of respiration, particularly in its amplitude, was observed. There was no case in which respiration was arrested. The initial dose for an appropriate response varied from 1-10 gamma. With subsequent intracisternal injections, increasingly larger doses were required to produce a similar rise of the blood pressure. The rise became less sharp but more prolonged with successive injections. Sectioning the vagi and atropinizing the animal abolished the intravenous but not the intracisternal response to acetylcholine. The intravenous response was invariably depressor.

Acetylcholine applied directly to the floor of the exposed IVth ventricle also produced a rise in blood pressure. The site of stimulation is now under investigation.

# Missouri Section

*Washington University Medical School, May 15, 1935.*

## 8113 C

### Influence of Point at which Ventricle Contacts Thoracic Wall on Form of the Electrocardiogram.

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*From the Department of Medicine, Washington University School of Medicine, St. Louis, Missouri.*

The recent experiments of Katz,[1] Wilson,[2] and of Kountz, Prinzmetal and Smith[3] have indicated that the point at which the heart contacts the thoracic wall may play an important part in determining the character of the electrocardiogram. Especially the work of the latter authors has suggested that the point of the ventricle in contact with the chest wall, as well as the point on the chest wall where this contact is made, may have a profound influence on the resulting curves. It has also served to show that it is extremely difficult in the animal body to change the contact of the heart without changing its electrical axis. Since the influence of these changes has not been studied separately, the following method was devised.

A dog heart-lung preparation (Starling) was set up and removed from the dog's body. The thorax of a second dog, just dispatched, was opened anteriorly and the thoracic viscera left *in situ*. The esophagus of the heart-lung preparation was connected to a point 4 cm. to the left of the sternum on the anterior chest wall. On the heart-lung preparation a wire electrode which could be moved from place to place on either ventricle, was connected by a second wire to the esophagus in the opened thorax. In this way, a cardioelectric impulse arising in the heart-lung preparation was conveyed to the dog's body and recorded on the electrocardiograph by the 3 usual leads attached to the dog's body.

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[1] Katz, L. N., and Ackerman, W., *J. Clin. Invest.*, 1932, **11**, 1221.

[2] Wilson, F. N., Johnson, F. D., and Barker, P. S., *Am. Heart J.*, 1934, **9**, 472.

[3] Kountz, W. B., Prinzmetal, M., and Smith, J., *Am. Heart J.* (To be published.)

It was found that contact could be made with either ventricle of the heart-lung preparation without changing greatly the character of the normal QRS impulse. In the complexes the initial deflection of the galvanometer was always in the same direction. This was followed by a second deflection, which, when the left ventricle of the heart preparation was in contact, was tall (3mm.). When the right ventricle was in contact it was small. The second deflection of the galvanometer was always opposite in direction to the first; the following "T" wave was always in a direction opposite to its preceding second deflection.

With the contact on the same ventricle of the heart-lung preparation, extrasystoles were produced by an interrupted current applied to various parts of that ventricle. Thus, the contact on the same ventricle in different relationship to points of origin of the extrasystoles gave the same type of curve in each instance. Contact of the opposite ventricle, however, yielded curves, the reverse of those obtained before in leads one and three. A definite exception to this was noted when the point of stimulation of the heart was close to the septum in that a complete reversal of the curves did not occur upon changing the contact from one ventricle to another.

It was noted further that the voltage in the resulting curves, both for normal complexes and extrasystoles, was greatest when the apex of the heart was contacted, lowest when the right ventricle was contacted, and intermediate when the left ventricle was contacted.

The right and left bundles of the conducting system were then severed. When the right bundle was cut the electrocardiographic curve characteristic of bundle branch block was best seen when there was direct contact with the right ventricle. The evidence of bundle branch block was seen but poorly in some experiments and not at all in others, when the contact was through the left ventricle of the heart-lung preparation, even though simultaneous records of the right ventricle gave curves showing bundle branch block. When the left bundle was cut, changing the contact from the left to the right ventricle changed the direction of the curve from an upright deflection in lead one and down in lead three to down in all leads. Contact through the right ventricle did not modify the curve of bundle branch block as far as duration of the complex was concerned.

The results of these experiments indicate that change in the point of contact of the heart may greatly modify the electrocardiogram, particularly in the case of bundle branch block and extrasystoles, even though the axis of the cardioelectrical impulse remains unchanged. Results when both ventricles are in contact serve to emphasize that the character of the electrocardiogram is influenced more by the left than by the right ventricle.

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### 8114 C

## Effects of Human Anterior Pituitary Gland on Sex Organs and Thyroid of the Guinea Pig.*

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*From the Department of Pathology, Washington University School of Medicine, St. Louis.*

Two types of effects of anterior pituitary gland on the sex organs can be distinguished.[1] The first type is represented by the anterior pituitaries of cattle, hog and sheep. Extracts from these glands cause in the guinea pig a rapid atresia of follicles, a slight production of interstitial gland in the medulla of the ovary and in some atretic follicles a moderate enlargement of the theca interna and sometimes also of the connective tissue which organizes the central cavity of the follicles. Occasionally small or medium sized pseudocorpora lutea may develop. The thyroid gland undergoes a marked hypertrophy. If instead of injection of extract, pieces of anterior pituitary from these species, each one about the size of a pea, are implanted, similar effects are obtained. On the other hand, implantation of pieces from rabbit, rat, and cat anterior pituitary induces in addition to the marked luteinization of the theca interna and the increased production of interstitial gland in the medulla of the ovary an early maturation and luteinization of the follicular granulosa, and the subsequent transformation of this hypertrophic granulosa and theca interna into lutein bodies and pseudocorpora lutea. This occurs if larger follicles are thus affected; somewhat smaller follicles including those which are in process of atresia are changed into interstitial gland bodies in which the theca interna and perhaps also the granulosa undergo luteinization. Daily im-

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*These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Loeb, Leo, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 642, 1128; *Endocrinology*, 1932, **16**, 129; Loeb, Leo, and Friedman, Hilda, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 741.

plantation of one or 2 guinea pig anterior pituitary glands causes merely a growth of follicles to large size with subsequent granulosa maturation and formation of strands of interstitial gland in the medulla. It does not as a rule induce hypertrophy of the thyroid gland, whereas similar quantities of rat and rabbit anterior pituitaries are effective in this respect. The action of extracts prepared from anterior pituitaries of the second (rabbit) group in the same way as extracts from the first group has not yet been studied.

It was of interest to determine what would be the effects of human anterior pituitary and whether this kind of gland belongs to one of these 2 groups. Zondek and Aschheim in a few implantations of human anterior pituitary glands into mice,[2] noticed enlargement of the ovaries, uterus and occurrence of estrus. They concluded that both hormones A and B are present in human anterior pituitary glands. Schultze-Rhonhof and Niedenthal[3] transplanted pieces of human anterior pituitaries into mice and rats and obtained similar results. They noted keratin formation in the vagina, and the formation of corpora lutea and pseudocorpora lutea in the ovaries. However, these investigators did not yet distinguish between different types of anterior pituitaries, because they used as test animals mice and rats which are not suitable for the analysis of finer differences in the action of the anterior pituitary, probably because in these species the granulosa of follicles is much more resistant than it is in the guinea pig.

We implanted human anterior pituitary glands obtained at autopsies into 9 female guinea pigs with an initial weight varying between 180 and 205 gm. As a rule, each animal received daily one-fourth of a human anterior pituitary for 4 consecutive days; examination took place on the fifth day. A tenth guinea pig received daily one-fourth of a gland for 8 consecutive days, 2 glands being used altogether; the last animal received daily one-half of an anterior pituitary gland from 5 different anterior pituitaries, the gland from a different case being inoculated each day and the examination taking place on the sixth day. Three additional guinea pigs were injected daily with 1 cc. of an extract prepared from a larger number of human anterior pituitaries in exactly the same way as our acid extract of cattle anterior pituitary. These animals received 2, 4 and 8 injections respectively of this extract. In all cases the ovaries were cut into complete serial sections and sections of uterus, vagina, mammary gland, thyroid and adrenal gland were likewise studied.

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[2] Zondek, B., *D. Hormone des Ovariums und des Hypophysen vorderlallen*, Berlin, 1931.

[3] Schultze-Rhonhof, F., and Niedenthal, R., *Zentralblatt f. Gynaekol.*, 1928, **52**, 1892.

In all essential respects the same results were obtained in all these cases, although some minor differences were observed. The weight of the guinea pigs remained in the majority of cases approximately stationary during the experiment; in 3 cases, there was a loss of 10 to 15 gm. and in 2 other cases a slight gain; in the animal receiving 8 injections the weight increased from 195 to 250 gm.

The changes in the ovaries induced by human anterior pituitary gland are as follows: 1. There is a marked tendency of the granulosa of follicles to mature and to luteinize; this applies not only to large but even to small or medium sized follicles.

2. Associated with this process of luteinization in the granulosa is a luteinization of the theca interna. Somewhat later connective tissue and capillaries may grow through the enlarged granulosa and into the central cavity of the follicles. The degree of maturation and luteinization of the granulosa varies in different follicles; it is usually less marked than the luteinization of the theca interna. This leads to an hypertrophic type of follicular atresia.

3. The connective tissue growing into the center of the cavity of follicles during the process of ordinary atresia and especially that surrounding the egg may enlarge and assume the character of epithelioid tissue.

According to the size of the follicles thus affected and the rapidity and intensity of luteinization of the granulosa, either pseudolutein bodies or luteinizing atretic follicles develop. The large pseudolutein bodies become gradually transformed into pseudocorpora lutea. Around follicles in the later stages of connective tissue atresia a considerable amount of luteinized tissue develops; how far this consists solely of enlarged theca interna or also of remnants of luteinized granulosa is difficult to decide.

4. In the medulla of the ovary strands of large interstitial gland tissue develop; the degree of their development corresponds, on the whole, to the degree of luteinization of the theca interna.

The difference between the effects of anterior pituitaries obtained from different cases was not striking; however, some differences were noted as to the presence of large follicles, the greater or lesser tendency of follicles to mature and the number of pseudolutein bodies and pseudocorpora lutea which developed. In one case in which a guinea pig received 8 injections of extract of human anterior pituitary we found what were apparently true corpora lutea, in which we could not discover any retained eggs. Extracts of human anterior pituitary acted essentially like implanted gland tissue. We may therefore conclude that the process of preparing the extract does not cause a destruction of the hormone acting on the granulosa of the follicles.

Corresponding to the ovarian effects were the changes in the secondary sex organs. In the vagina in a number of cases there was a moderate proliferation of the cuboidal epithelium, leading to the formation of 2 or 3 layers covered by a layer of vacuolar cylindrical cells. As pointed out previously a complete development of squamous and keratin forming epithelium does not take place under these conditions, notwithstanding the presence of a number of mature follicles. In contrast with this relative inactivity of the vagina under these conditions is the active proliferation in this organ which we find at the time of normal estrus, when a single mature follicle readily calls forth very active proliferation and keratinization. We may assume that the luteinized theca interna tissue inhibits the full effect which during normal estrus the mature granulosa exerts on the vaginal epithelium. In still other cases the proliferation in the vagina is lacking altogether; this is found especially in instances in which large mature follicles are absent. However, these relations are not always clear cut; it is probable that simultaneous action of the mutually inhibiting systems, namely mature granulosa and luteinized theca interna, introduces certain complications.

The uterine mucosa has a tendency to form predecidual tissue, especially in cases in which pseudolutein bodies and pseudocorpora lutea are prominent; but here also certain variations occur. In the mammary gland, we find in the majority of cases more or less proliferation which may be associated with signs of secretion; but the latter may be noted also in cases in which proliferation is lacking.

The thyroid gland was in all cases hypertrophic, although the degree of hypertrophy varied in different cases. It was usually quite marked, but in some instances only moderate. It was least marked in the animal which received 8 injections of extract; in this guinea pig correspondingly the gain in weight was greatest. As to the adrenal cortex, accurate counts of the number of mitoses present were not made; but it was evident that in quite a number of animals the proliferative activity in this organ was much increased.

*Conclusion.* The human anterior pituitary exerts about the same effects in the guinea pig as the anterior pituitary glands of rabbit and rat and it differs markedly in its effects from the anterior pituitaries of cattle, hog and sheep. This is true especially as far as the effects on ovaries and secondary sex organs are concerned; both types of anterior pituitaries produce a stimulation of thyroid and adrenal glands. Injection of extracts of human anterior pituitaries act in about the same way as implantations of pieces of gland tissue.

## 8115 C

## Effects of Cattle Anterior Pituitary Extracts and KI on Liver Glycogen in Guinea Pigs.*

RAYMOND HOLDEN and ERIC W. THURSTON. (Introduced by Leo Loeb.)

*From the Department of Pathology, Washington University School of Medicine, St. Louis.*

Silberberg[1] has shown that oral administration of KI inhibits the hypertrophic changes in the thyroid gland which are produced by injections of extracts of anterior pituitary gland of cattle. A similar inhibiting effect was shown by Siebert and Thurston to be exerted by KI administration in respect to the rise in metabolism which otherwise would have been produced by injections of such extracts of anterior pituitary. We now wished to determine whether KI exerts an inhibiting effect on the changes in carbohydrate metabolism which, according to Eitel and Loeser[2] as well as Holden,[3] are called forth when extracts of cattle anterior pituitary gland are injected into guinea pigs. These changes consist in a marked diminution in the liver glycogen and an increase in blood sugar which Holden finds is of a very transitory character.

Male guinea pigs (190-220 gm.) kept under optimum conditions were used in the majority of tests. In certain cases females were used. The experiments were divided into 2 parts. In the first part, 13 guinea pigs were injected with anterior pituitary extract and simultaneously fed KI. In the second part, 14 guinea pigs were given KI alone. The amount of extract of cattle anterior pituitary gland injected daily intraperitoneally was 1 cc. Two or more animals were injected for each period, namely 2, 4, 6, and 8 days. For 4 days previous to the first injection and continuing throughout

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* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Holden, Raymond F., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 773.

[2] Eitel, H., and Loeser, A., *Arch. Exp. Path. u. Pharm.*, 1932, **167**, 381.

[3] Shaffer, P. A., and Somogyi, M. J., *Biol. Chem.*, 1933, **100**, 695.

the period of injections each animal received daily 0.05 gm. of KI *per os*. Twenty-four hours following the last injection, the animals were killed by a blow on the head, and immediately following death blood from the heart and 2 pieces of liver weighing from 0.5-1 gm. each were removed from each guinea pig. Blood sugars were determined according to the Shaffer and Somogyi[3] method. Duplicate glycogen determinations were carried out by the modified Pflueger[4] method. As the normal values for glycogen and blood sugar we accepted the amounts determined by Holden in his earlier experiments.

Administration of KI did not have any noticeable effect on the decrease in liver glycogen produced by injections of anterior pituitary extract. The minimum values for glycogen were obtained after 6 injections had been given; this was followed by an insignificant rise following the eighth injection. The average amounts obtained in 2 or more guinea pigs are shown in Fig. 1. Normal values in untreated animals according to Holden are 2540 mg. % and according to Eitel and Loeser 2590 mg. %. The administration of KI *per os* does not therefore modify appreciably the effects of administration of anterior pituitary extract on the liver glycogen. (See Fig. 1.)

In a control experiment, a number of guinea pigs were given

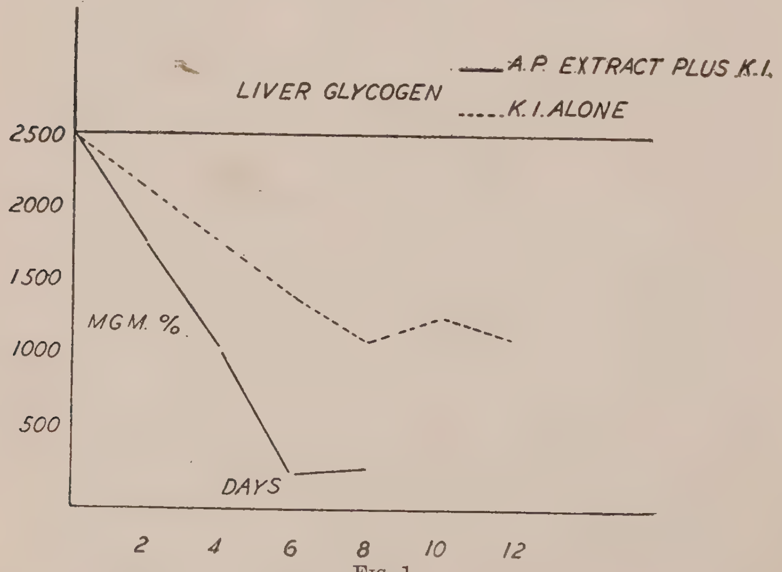

FIG. 1.

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[4] Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 483.

daily doses of 0.05 gm. of KI alone by mouth, the total quantities of KI received by these animals being the same as the total quantities given simultaneously with anterior pituitary extracts in the preceding experiment. The effects of the administration of KI alone are also represented on the chart. It is noted that KI, as such, produces a reduction in liver glycogen, but the reduction is less in this case than when KI is given in conjunction with anterior pituitary extracts; even after 12 days' feeding of KI the glycogen failed to diminish in amount to below 1140 mg. %. Whether this reduction is due to a stimulation of the thyroid has not yet been decided. In other species than the guinea pig such an effect has not yet been observed. Thus in white mice, Romeis and Zwehl[5] found no effect on liver glycogen after administration of sodium iodide. Likewise, Coggeshell and Greene,[6] using albino rats, were not able to demonstrate a change from the normal in liver glycogen after administration of inorganic iodides.

In neither of our series of experiments did we find significant changes in the blood sugar content.

*Conclusions.* 1. KI given *per os* previously to and simultaneously with intraperitoneal injections of anterior pituitary extracts of cattle fails to modify the effect of the extracts on the liver glycogen. 2. Oral administration of KI alone may cause a moderate reduction in liver glycogen.

### 8116 P

## Deleterious Effects of Local Application of Staphylococcus Bacteriophage.*

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*From the Department of Bacteriology and Immunology, Washington University Medical School, St. Louis.*

During the study of the therapeutic value of specific bacteriophage in experimental staphylococcus skin infections in rabbits it was observed that certain strains of bacteriophage, instead of promoting the healing, have caused a temporary intensification of the local

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[5] Romeis, B., and Zwehl, T., *Klin. Woch.*, 1925, **4**, 703.

[6] Coggeshell, H. C., Greene, J. H., *Am. J. Physiol.*, 1933, **105**, 103.

* This work was supported from a grant by the Rockefeller Foundation to Washington University for research in science.

reaction. Suspecting that this effect might have been due to the presence of the "Reynals Spreading Factor" in the bacteriophage lysate, the following experiments were performed.

An 18-hour agar slant culture of non-invasive staphylococci was suspended in 10 cc. of sterile distilled water, diluted with an equal volume of broth, and 1 cc. of the resulting suspension was injected intracutaneously into two sites on each flank of each of the 5 rabbits. The hair on both flanks was removed by means of barium sulphide 2 days previous to injection. The sites of the injections in 4 of the rabbits were covered by wet dressings consisting of a piece of folded gauze on top of which was placed a small pad of absorbent cotton which in turn was covered with gauze. A circular piece of adhesive tape with a hole in the center was used to hold the dressing in place. Through the hole in the adhesive tape the dressings were saturated twice daily with (1) broth (Rabbit No. 356) (2) bacteriophage propagated upon the homologous non-invasive strain of staphylococci (Rabbit No. 358), (3) bacteriophage propagated upon a highly invasive strain (Rabbit No. 357) and (4) autolysate of the invasive strain prepared according to Duran-Reynals,[1] (Rabbit No. 359). The fifth rabbit (No. 354) receiving the injection of the culture served as control, and was left without any dressings.

The dressings were removed once every day and the size of the lesions measured in each case as follows: The outline of the entire area involved in the reaction at each site of infection was traced with India ink on a strip of cellophane laid over the lesion, and subsequently measured with a planimeter. The area of the individual lesions in each rabbit was remarkably constant. The daily measurements of the infected areas thus permitted to compare the evolution of the lesions in the respective rabbits as depending on the nature of the solution applied to the dressing.

Each of the values plotted on Fig. 1 represents the average area in square centimeters of the 4 lesions in each of the rabbits.

Identical findings were obtained in another similar experiment, the results of which are plotted on Fig. 2. In this experiment rabbit No. 361 served as control; the dressings of rabbit No. 360 were saturated with broth, rabbit No. 555 with bacteriophage propagated on a strain of invasive staphylococcus, rabbit No. 356 with bacteriophage propagated on a strain of non-invasive staphylococcus and rabbit No. 547 with autolysate of invasive staphylococcus.

The results of these experiments show that (1) the final healing

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[1] Duran-Reynals, F., *J. Exp. Med.*, 1933, **58**, 161.

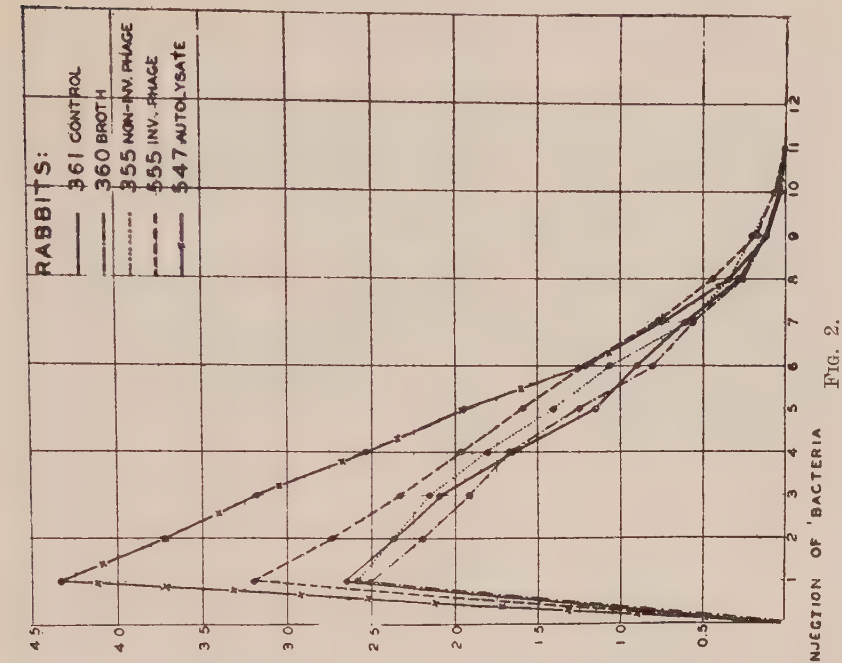
RABBITS:
361 CONTROL
360 BROTH
355 NON-INV. PHAGE
355 INV. PHAGE
347 AUTOLYSATE
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 2.

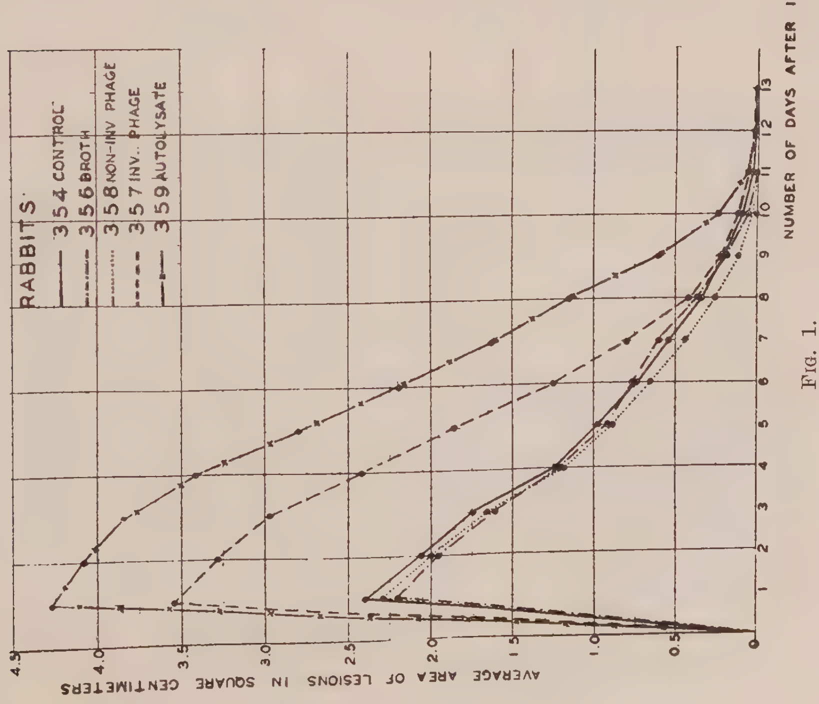
RABBITS:
354 CONTROL
356 BROTH
358 NON-INV PHAGE
357 INV. PHAGE
359 AUTOLYSATE
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
NUMBER OF DAYS AFTER INJECTION OF BACTERIA
AVERAGE AREA OF LESIONS IN SQUARE CENTIMETERS

FIG. 1.

of all of the lesions took place at about the same time irrespective of the type of dressing applied, that (2) the "Reynals Factor" markedly enhanced the size of the lesions, that (3) the bacteriophage lysate obtained with the highly invasive strain, presumably containing "Reynals Factor", definitely increased the size of the lesions as compared to the controls, and that (4) the bacteriophage propagated on the homologous non-invasive strain though causing no enhancement, produced no therapeutic effect.

Thus it is apparent that if the culture of staphylococcus used in the preparation of the bacteriophage happens to be of the invasive type the local application of the lysate thus prepared may result in undesirable local reactions.[2] Similar experiments performed with highly invasive staphylococcus are now in progress, and will be reported subsequently.

## 8117 P

## Morphology of Bacillus of Rat Leprosy.*

E. V. COWDRY AND L. F. HEIMBURGER.

*From the Anatomical Laboratory, Washington University, St. Louis.*

Ziehl-Neelson stained sections of tissues prepared by the new freezing and drying technique (Gersh,[1] Bensley and Gersh[2] and Scott[3]) show bacilli of smoother outlines and much less granular in consistency than those brought to light by the same stain after fixation in the ordinary way in 10% formalin in absolute alcohol or Regaud's fluid. Since the new and the old methods give such different results with exactly the same material (liver nodules of rats, kindly sent to us by Drs. E. B. McKinley and E. L. Walker) both cannot be equally true to life. The freezing and drying technique involves instantaneous freezing of tissue by plunging into liquid air and dehydration *in vacuo* while still frozen. No fixative is required, no alcohol for dehydration and no xylol for clearing. Bensley and Gersh have great faith in it. They conclude, because well-formed Nissl bodies can be stained in sections thus prepared, that the said bodies must exist in living nerve cells despite the fact that they have never been seen therein. Thus far we have not compared the homogeneous looking bacilli of the new technique and the more broken up organisms observed in preparations made by standard methods with bacilli of rat leprosy in living cells. We have, however, made a parallel study of human tubercle bacilli in visceral lesions (for which we are indebted to Dr. Ralph S. Muckenfuss). In this case also, the bacilli were distinctly less granular after the freezing and drying technique than in tissues preserved by immersion in 10% formalin in absolute alcohol, straight 10% formalin, formalin-Zenker or Regaud's fluid. These observations suggest, either that there is some unsuspected flaw in the freezing and drying method, or that the bacillus of rat leprosy and the human tubercle bacillus as they exist in the tissues are less granular than is commonly supposed.

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[2] King, W. E., Boyd, Jr., D. A., and Conlin, J. H., *Am. J. Clin. Path.*, 1934, **4**, 336.

* Aided by a grant from the Leonard Wood Memorial.

[1] Gersh, I., *Anat. Rec.*, 1932, **53**, 309.

[2] Bensley, R. R., and Gersh, I., *Anat. Rec.*, 1933, **57**, 369.

[3] Scott, G. H., *Protoplasma*, 1933, **20**, 133.

## 8118 P

## Effects of Extract of Cattle Anterior Pituitary Gland on Endochondral Ossification in Young Guinea Pigs.

MARTIN SILBERBERG. (Introduced by Leo Loeb.)

*From the Department of Pathology, Dalhousie University, Halifax, N. S.*

It has been established that the anterior pituitary gland enhances bodily growth as a whole, but it is not yet known whether it has a specific effect on the growth of cartilage and bone and, if this should be so, wherein the effect consists. We wished, therefore, to study the action of anterior pituitary gland extract on the epiphyseal cartilage and bone formation in young not yet fully grown guinea pigs.

About 30 guinea pigs (fall and winter animals) on the average weighing 130 to 220 gm. were injected with from 1 to 1½ cc. extract[1] daily. Additional guinea pigs, of similar weight, were not injected and served as controls. The injections were continued up to 21 days. After 4, 6, 11, 14, and 21 days, the animals were killed and tibia and fibula were used for the study of the epiphyseal line. In each case the specimen, as a whole, was fixed in 10% formalin; then put into absolute alcohol and subsequently transferred into ether for 2 days, in order to remove the fat from the bone-marrow. The bones were then washed in running water and decalcified incompletely in order to preserve and demonstate the calcium salts. In accordance with Pommer's method, they were kept about 3 to 8 weeks in Mueller's solution, until they had become sufficiently soft for cutting; subsequently they were embedded in celloidin and stained with hematoxylin-eosin or hematoxylin-Van Gieson.

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[1] Loeb, Leo, and Basset, R. B., Proc. Soc. Exp. Biol. and Med., 1929, **26**, 860. Silberberg, M., *Virchow's Arch.*, 1933, **289**, 201.

After 4 injections, a stimulation of the growth of cartilage and bone can be seen at the epiphyseal line. The undifferentiated mesenchymal cells have become mobilized and transformed into chondrocytes and osteocytes. The columnar resting cartilage cells are increased in size and number, mitotic figures are found everywhere and are more numerous than under normal conditions, the stroma between the cells correspondingly is much diminished in amount. Likewise in the layer of the vesicular cartilage, hyperplasia and hypertrophy are noted. After 7 to 10 injections, the epiphysis as a whole, has become more narrow in comparison with that of the control animals. However, in addition, we notice now pronounced disturbances of endochondral ossification. The epiphyseal line has become completely calcified and is therefore in the majority of cases closed in contradistinction to the normal epiphyseal line in the controls. Furthermore, the layer of the indifferent mesenchymal cells and of the columnar cartilage cells has become irregular, the cytoplasm of the latter having taken up calcium salts. The vesicular cartilage cells are hypertrophic and in some cases may show giant cell formation; the number of mitotic figures is here increased. On the other hand, changes on the part of the osteoclasts were not noted. After the growth of the cartilage has thus been stimulated, this tissue undergoes calcification and is quickly replaced by bone tissue. This process takes place in the cartilage equally on the side of the epiphysis and of the diaphysis. Corresponding growth changes as well as disturbances of the endochondral ossification are seen also in the other long bones.

After 14 injections the epiphyseal line was not yet completely calcified in about 20% of the animals, in which latter the effects observed at this time were the same as those usually seen after 4 injections. After 21 injections, increase in size and proliferation of the various kinds of cartilage cells are still more pronounced, and the epiphyseal line is now completely closed.

*Conclusions.* Injections of acid extract of anterior pituitary of cattle have a stimulating effect on the growth of cartilage and bone of growing guinea pigs. The various layers of the cartilage cells become hypertrophic and hyperplastic, and are subsequently quickly calcified and replaced by bone. The epiphyseal line undergoes, therefor, premature closure under the influence of the extract.

## 8119 C

## Effect of Various Anterior Pituitary Gland Preparations on Thyroidectomized Guinea Pigs.

K. C. MORRIN AND LEO LOEB.

*From the Department of Pathology, Washington University School of Medicine, St. Louis.*

It has been shown that following repeated injections of cattle anterior pituitary extract a very extensive atresia of follicles takes place in the ovary of the guinea pig, which is in contrast to the growth and maturation of follicles that occurs after implantation of anterior pituitary glands of rabbit, rat and guinea pig.[1] A similar, though less pronounced atresia of follicles may be produced through undernourishment of guinea pigs (development of hypotypical ovaries).[2] It was of interest to determine whether the thyroid gland which is rendered strongly active by these extracts is concerned in this effect on the ovarian follicles. This question was of interest, because it has been shown that some of the effects of anterior pituitary extracts are mediated by the thyroid gland; and furthermore, that thyroid hormone increases basal metabolism and may lead to a loss of weight in the animal in which it is present in excess.

We first carried out control experiments in which we thyroidectomized 21 guinea pigs with an original weight varying between 175 and 205 gm. (except one guinea pig which weighed 260 gm.). Autopsies were performed at intervals between 6 and 38 days following thyroidectomy. The thyroidectomy was complete in 13 animals, while in the remaining 8 some remnants of the thyroid were found. The operation caused a loss in weight or prevented a normal gain, especially in guinea pigs with an original weight less than 190 gm. The heavier animals had an initial loss of weight for the first few post-operative days followed by a rapid gain. It was found that complete thyroidectomy does not interfere with the normal development of the ovarian follicles and of the secondary sex organs when the weight remains normal. Loss in weight may cause an incomplete development of the follicles (hypotypical ovaries); but this condition is less marked than that caused by injections of cattle anterior pituitary extracts. The latter cause a very pronounced atresia of follicles even in guinea pigs in which the general state of nourishment would permit growth of the follicles to medium and large size. In completely thyroidectomized guinea pigs spontaneous ovulation may take place when a weight approximating 300 gm. has been reached. In one of these animals, a placental body developed in the ovary as the result of parthenogenetic development of an ovum. This condition was probably independent of the preceding thyroidectomy. The 8 guinea pigs in which the thyroidectomy had been incomplete showed no loss of weight following the operation, presumably because in all these animals the initial weight was relatively high. The ovarian follicles and the secondary sex organs behaved in the normal manner. The remnants of thyroid tissue underwent compensatory hypertrophy in the course of time.

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[1] Loeb, Leo, Proc. Soc. Exp. Biol. and Med., 1932, **29**, 642, 1128; *Endocrinology*, 1932, **16**, 129.

[2] Loeb, Leo, *Biol. Bull.*, 1917, **32**, 91.

Sixteen thyroidectomized guinea pigs were injected with cattle anterior pituitary extract. In 9 of these animals, no remnants of thyroid gland were found at autopsy, while in 7 the thyroidectomy had not been quite complete. The weight of the guinea pigs at the time of operation varied between 190 and 205 gm., with the exception of one animal which weighed 180 gm. Following the operation, an interval varying between 6 and 37 days was permitted to elapse before the injections of extract were commenced. During the first 10 days following the operation, the weight of the guinea pigs remained stationary or showed a slight loss with a subsequent gain in weight of about 50 to 90 gm. The period of injections during which one injection of extract was given daily, varied between 2 and 11 days. During the first 4 days of injection, there was in some animals a loss in weight amounting to between 5 and 25 gm.; the others gained 10 to 35 gm. The amount of extract injected daily, varied from 1½ cc. to 3 cc.

The results of the injection of acid extract were typical. A very extensive atresia of follicles occurred soon after the beginning of the injections and only in very small, or small to medium-sized follicles did the granulosa remain preserved, and even in these follicles there was a tendency for the granulosa cells to dissolve. The follicles reached medium to somewhat larger size before atresia set in, in only 2 cases. In some of the atretic follicles, the theca interna and part of the connective tissue growing into the follicular cavity luteinized, and in some cases smaller pseudolutein bodies and pseudocorpora lutea developed. In one of the larger animals, in which ovulation had taken place prior to the commencement of the injections of extract, these corpora lutea underwent retrogressive changes. In the medulla of the ovary rudimentary strands of interstitial gland were seen quite commonly. Vagina, uterus and mammary gland were in a resting condition; but in the uterine mucosa some activity was noticeable in some cases, leading to the formation of predecidual connective tissue in one case. The activity in the uterine mucosa seemed to be correlated with the luteinization processes which took place in the ovary.

We may then conclude that in thyroidectomized guinea pigs extract of cattle anterior pituitary gland has the same effects on the ovary as in normal animals and the effects of this extract on the ovary are not mediated by the thyroid gland but take place independently of the latter.

The same applies to the effects obtained by implanting anterior pituitary glands of rabbit, rat and guinea pig into young female guinea pigs. In 8 guinea pigs such glands were implanted daily during a period of 4 or 5 days. In 2 of these animals no remnants of thyroid were found at autopsy, while some small remnants of isthmus were found in the others. In all of them the typical effects on the granulosa and theca interna were obtained, *viz.,* large mature follicles, pseudolutein bodies and pseudocorpora lutea as well as interstitial gland bodies developed. In some cases, a premature luteinization of the granulosa began in the follicles which had not reached full size. The results varied typically in accordance with the kind of anterior pituitary which was implanted and the amount of gland tissue used, as has been described previously. Here also thyroidectomy did not modify the results in a noticeable manner.

*Conclusion.* The changes which extract of cattle anterior pituitary gland and anterior pituitary of rabbit, rat and guinea pig exert on the ovary and on the secondary sex organs take place independently of the thyroid gland. It is due to a direct effect on the ovary and is not noticeably modified by the lack of thyroid function.

## 8120 P

# Mineral Salts of the Nucleus.*

GORDON H. SCOTT.

*From the Department of Anatomy, Washington University School of Medicine, St. Louis.*

Hueper[1] indicated that in certain tumor cells the correlation between chromatin material in stained sections and the mineral residue after microincineration was not as close as might be supposed from my studies of similar and of other material (Scott[2, 3, 4]). From Hueper's extension of his remarks to other classes of material, protozoa, etc., studied by me it might be inferred that he believed his observations on tumors to be of general significance. Cowdry,[5] Rector and Rector,[6] Policard,[7] Kruszynski[8] and others have amply demonstrated with various kinds of mammalian tissues, both normal and pathological, that my observations are not unique. Examination of a large series of human tumor and cancer tissues of many sorts collected and described in part by Olch[9] served to confirm in detail my previous statements made with regard to the ash-bearing chromatin material. Studies of an incinerated series of sections of embryos (chick, rat, mouse and cat) have given additional confirmatory evidence.

By taking advantage of 2 recently developed technical procedures a means was found of experimentally checking these observations. Tissues (liver and testis) of rats, guinea pigs and cats were subjected to ultra-centrifuging, as described by Beams, King and Risley,[10] at 100,000 times gravity. The centrifuge cup with its contents was frozen in liquid air and placed in a specially designed

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* Aided by grants from the Cyrus M. Warren Fund of the American Academy of Arts and Sciences; the International Cancer Research Foundation; and the National Research Council.

[1] Hueper, W. C., *J. Lab. and Clinical Med.*, 1934, **19**, 1293.

[2] Scott, G. H., and Horning, E. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 708.

[3] Scott, G. H., and Horning, E. S., *Am. J. Path.*, 1932, **8**, 329.

[4] Scott, G. H., *Am. J. Anat.*, 1933, **53**, 243.

[5] Cowdry, E. V., *Am. J. Path.*, 1933, **9**, 149.

[6] Rector, L. E., and Rector, E. J., *Am. J. Path.*, 1933, **9**, 587.

[7] Policard, A., *Bull. d'Histol. Appliq.*, 1933, **10**, 313.

[8] Kruszynski, J., *Bull. Acad. Polonaise d. Sci.*, 1934, **3**:B II, 105.

[9] Olch, I. Y., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 511.

[10] Beams, H. W., King, R. L., and Risley, P. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 181.

low temperature vacuum dehydrator. All water was removed while the tissues were kept frozen at —32°C. Tissues so treated were embedded in paraffine after the method advised by Gersh[11] and Bensley.[12] Serial sections were made and alternate ones stained by several methods; the remaining sections were incinerated and examined with the darkfield microscope.

Stained sections of centrifuged tissues show the chromatin material packed at the centrifugal pole of the nucleus, against the nuclear membrane.

Incinerated sections reveal a dense mass of whitish ash at the side of the nucleus corresponding in position to the stained chromatin of the control slides. Very little mineral residue is visible in the clear portion of the nucleus.

By fortunate circumstances it was possible to obtain a good specimen of human testis prepared by the Gersh-Bensley method. Incinerated sections when compared with the stained controls revealed the fact that the chromosomes left a clearly recognizable ash picture of themselves. This would seem to leave little doubt as to the location of mineral salts in the nucleus and its components.

When sections of frozen-dehydrated tissues treated with petroleum ether to remove the paraffine are allowed to become moist by standing in air at room temperature, then dried at 40-60°C. and incinerated they show nuclei which contain diffuse ash. Free hand sections of frozen dehydrated tissues not infiltrated with paraffine given the same treatment show essentially the same picture. This procedure is not too unlike the basic manipulations of the techniques used by Schultz-Brauns[13] and by Hueper (loc. cit.) to permit the suggestion that it may account for the results obtained.

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[11] Gersh, I., *Anat. Rec.*, 1932, **53**, 309.

[12] Bensley, R. R., *Anat. Rec.*, 1933, **58**, 1.

[13] Schultz-Brauns, O., *Z. f. wissensch. Mikr.*, 1931, **48**, 161.

# Illinois Section

*Loyola University Medical School, May 21, 1935.*

## 8121 C

### An Attempt to Infect Vitamin-Deficient Animals with Poliomyelitis Virus.

ORAM C. WOOLPERT AND JAMES A. HARRISON. (Introduced by N. Paul Hudson.)

*From the Department of Hygiene and Bacteriology, the University of Chicago.*

The great need for further experimental work in poliomyelitis and the fact that the monkey has been the only responsive experimental animal have evoked numerous attempts[1] to adapt the virus to smaller laboratory animals. The most recent of these was made by Brodie and associates,[2] who claimed that mice were successfully infected following reduction of their natural resistance to the virus by means of X-ray radiation.

We have approached the problem by using rats, mice and guinea pigs which were kept on a diet deficient in vitamins B and C. The diet consisted of casein (Harris Vitamin-B free) 20 parts, starch (Harris Vitamin-B free) 60 parts, purified butter fat 10 parts, agar-agar (Merck) 4 parts, and Osborne-Mendel Salt Mixture 4 parts. Young recently weaned animals were kept on this diet for a period of 10 to 25 days before inoculation and were maintained on the diet during the experimental period. They manifested marked symptoms of vitamin deficiency, such as weakness, shaggy fur, failure to gain weight or loss of weight, and sometimes cutaneous and mucous membrane lesions. Occasionally it was necessary to revert to a more complete diet for short periods (addition of yeast) to maintain life. Rats tolerated the management fairly well, mice not quite so well, and guinea pigs poorly.

Two preparations of virus were used: a chemically purified product, and a suspension of monkey cord which had been preserved in glycerin. Both were infectious for monkeys in the amounts used experimentally. The animals were inoculated under anesthesia, the rats and guinea pigs with 0.1 to 0.2 cc. intracerebrally and 1.0 cc. intraperitoneally, the mice with 0.05 cc. intracerebrally and 0.5 cc. intraperitoneally. Subinoculations were made in the same manner, brain and cord serving as the source of the subinoculation material.

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[1] Poliomyelitis, International Committee, Williams and Wilkins, Baltimore, 1932.

[2] Brodie, M., Goldberg, S. A., and Stanley, Phyllis, *Science*, **81**, March 29, 1935.

The purified virus was carried through 6 transfers in rats and 4 in mice, the crude virus preparation through 6 rat and 3 mouse passages. The crude virus was also inoculated into several adult guinea pigs and passed through 3 transfers in young guinea pigs. All of the transfers were made at 3-day intervals. Two or 3 animals were used at each stage, one being sacrificed for subinoculation material; the others were allowed to live for continued observation. The latter animals were killed and examined several weeks after inoculation or whenever intercurrent disease necessitated termination of the experiment.

In none of the animals were there symptoms suggestive of poliomyelitis as it is seen in man or monkey, and histological examination of the cord and brain tissue failed to show any changes referable to the disease. An acute inflammatory reaction was noted both grossly and microscopically in the meninges of one mouse. This was shown to be due to *Bacterium enteritidis*.

8122 C

## Fibrinolytic Activity of Hemolytic Streptococci from Scarlet Fever.

G. M. DACK, ORAM C. WOOLPERT, AND ARCHIBALD L. HOYNE.

*From the Department of Hygiene and Bacteriology, University of Chicago, and The Municipal Contagious Disease Hospital, Board of Health, Chicago.*

Tillett and Garner[1] have demonstrated an extracellular substance freely excreted by growing cells of beta hemolytic streptococci which has the property of dissolving solid human fibrin. They[2] have shown also that patients convalescing from acute hemolytic streptococcus infections develop a relative resistance to this fibrinolytic action. Their studies included 60 normal individuals and 11 patients with hemolytic streptococcus infections, 3 of whom had scarlet fever.

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[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[2] Tillett, W. S., Edwards, L. B., and Garner, R. L., *J. Clin. Invest.*, 1934, **13**, 47.

With the thought that there might be some correlation between the fibrinolytic activity of hemolytic streptococci in human infections and the clinical severity of the disease, we have studied 303 cases of scarlet fever occurring during the winter of 1934-35, and cared for at the Municipal Contagious Disease Hospital, Chicago. In 52 of these cases we have studied (1) the action of the patient's throat strain against a standard plasma-clot, (2) the reaction of the patient's plasma-clot to a standard strain, and (3) the action of the patient's strain on his own plasma-clot. In 47 of these cases 2 or more plasma specimens and corresponding cultures were tested, the interval between the taking of the first and second specimens varying from 10 to 39 days. In the remaining 251 cases only the fibrinolytic activity of the patient's strains was studied. Twenty-five of these represent cultures obtained from suppurative mastoids at the time of mastoidectomy, the others are throat cultures. In 70 of the latter group a second culture was taken about 2 weeks following the first.

The selection of patients in this study was made without regard for the clinical condition, except that in most instances only patients in the early stages of the disease, who had definite scarlatiniform rash at the time of the first culture, were chosen. First specimens were taken usually in the hospital receiving room or during the first or second day of hospitalization.

Clinical data on this series of patients were assembled after completion of the laboratory studies and about 3 weeks following admission of the last patient of the series to the hospital. Therefore, a small number of these patients are still under hospitalization because of complications and the mortality statistics might ultimately be slightly modified. Also in a few instances it is likely that patients listed as being uncomplicated at the time of discharge developed complications at home. Our clinical report is based only on hospital data.

The technic described by Tillett and Garner[1] was followed, using the whole plasma-clot rather than purified fibrin. A "standard" strain was selected which we found consistently dissolved the fibrin clot of the "standard" plasma in about 15 minutes. For the latter we used the freshly drawn blood of 2 normal subjects. This was found to remain very constant in reaction when repeatedly tested against the standard strain. During the course of the experiments a slight modification of procedure was made. In the first 99 cases we selected only a single hemolytic streptococcus colony from each of the blood agar plate cultures of patients' throats; in the remainder as many as 5 to 10 well isolated colonies were picked to the same tube of broth. Although in repeated tests in which 2 or more colonies were transferred to separate broth tubes the several strains gave identical results, we felt that a more representative test culture would be obtained by pooling several colonies.

The results of these studies may be summarized briefly:

*Fibrinolytic Activity of Patients' Strains.* There appears to be a definite statistical correlation between the activity of the patient's strain and the presence of complications (Tables I and II). All of the 25 strains cultured from suppurative mastoids were strongly fibrinolytic. Time for lysis of the standard plasma clot ranged from 15 minutes to more than 24 hours. In 45 of the 303 cases (15%) lysis did not occur within the 24-hour period of observation. This compares favorably with the 17% of 123 human strains of hemolytic streptococci which were found inactive by Madison.[3] (For purposes of statistical analysis we have considered these strains as lysing within 36 hours.)

TABLE I.
Fibrinolytic Activity of Cultures of Hemolytic Streptococci Isolated from Scarlet Fever Patients.

| Complications | Lysis time in hr. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0-½ | ½-1 | 1-2 | 2-3 | 3-4.5 | 4.5-6 | 6-9 | 9-12 | 12-24 | 24 plus |
| 0 | 12 | 23 | 26 | 26 | 18 | 6 | 2 | 2 | 9 | 34 |
| + | 3 | 5 | 11 | 8 | 6 | 2 | 2 | | | 4 |
| ++ | 5 | 4 | 8 | 2 | 7 | 2 | | | 1 | 6 |
| +++ | 2 | 1 | 7 | 1 | 4 | | | | | |
| ++++ | 7 | 5 | 4 | 1 | | | | | 1 | 1 |
| Fatal | 5 | 2 | 1 | 1 | | | | | 1 | |
| Mastoid | 8 | 9 | 4 | 4 | | | | | | |

Complications:
0 = none.
+ = rhinitis or moderate cervical adenitis.
++ = persistent rhinitis and cervical adenitis.
+++ = suppurative cervical adenitis, suppurative otitis media, catarrhal mastoiditis, persistent albuminuria.
++++ = suppurative mastoiditis, septicemia, multiple abscesses, acute hemorrhagic nephritis.

TABLE II.
Summary of Table I.

| Complications | Total cases | Average lysis time hr. | Median hr. | Mode hr. |
|---|---|---|---|---|
| 0 | 158 | 10.5 | 2.69 | 24 plus |
| + | 41 | 5.8 | 2.19 | 1-2 |
| ++ | 35 | 8.4 | 2.25 | 1-2 |
| +++ | 15 | 2.1 | 1.64 | 1-2 |
| ++++ | 19 | 3.7 | 0.75 | 0-½ |
| Fatal | 10 | 2.6 | 0.50 | 0-½ |
| Mastoid | 25 | 1.1 | 0.75 | ½-1 |

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[3] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1018.

A comparison of second cultures with first cultures in 70 cases reveals an increase of activity in 29 instances, a decrease in 20, and no change in 21. These differences may represent true changes in the strains, or cross infection in the hospital, or perhaps both factors may play a part.

*Resistance of Patients' Plasma-Clot to Fibrinolysis.* In the small series of 52 cases studied there was no consistent correlation of the reaction of the plasma with the presence of complications (Table III). In general the plasma of these patients, even early in the course of the disease, appeared relatively resistant to lysis, as compared with figures given by others[2, 4] for the plasma of normal individuals. Our studies suggest that the plasma of children under 5 years of age is highly susceptible to lysis, but the number of cases (6) is too small to warrant a conclusion. Of 47 instances where a second plasma was examined there was an increase in resistance to fibrinolysis in 13, including 5 complicated cases with one death. In 13 cases, including 3 with complications, resistance to fibrinolysis was diminished. In the remaining 21 there was no change.

TABLE III.
Analysis of 52 Cases with Complete Data on Cultures and Plasmas.

| Complications | No. of cases | Average time required for lysis, expressed in hours | | |
|---|---|---|---|---|
| | | Patients' first strain vs. standard plasma* | Patients' first plasma vs. standard strain* | Patients' first plasma vs. patients' strain |
| 0 | 32 | 11.52 | 12.17 | 14.45 |
| + | 13 | 2.5 | 10.25 | 15.25 |
| ++ | 2 | 0.75 | 36.0 | 19.0 |
| +++ | 2 | 1.12 | 1.12 | 4.75 |
| ++++ | 3 | 0.9 | 15.2 | 15.2 |
| Fatal | 2 | 0.37 | 4.75 | 4.75 |

(The 2 fatal cases are included in the ++++ group.)

*Control tests using standard plasma against standard strain gave a constant value of 0.25.

The reaction of the patient's plasma to his own strain did not seem to be correlated with the presence of complications.

Regarding an interpretation of the reaction of the patients' plasma, it should be stated that most of the more severely ill patients were given human convalescent scarlet fever serum intravenously early in the course of the disease; many of those critically ill were transfused, some of them repeatedly. We have no data bearing on the effect of these procedures on the patients' plasma.

*Conclusion.* In general, strains of hemolytic streptococci from complicated cases of scarlet fever are more actively fibrinolytic than strains from uncomplicated cases.

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[4] Hadfield, G., Magee, V., and Perry, C. B., *Lancet*, 1934, **226**, 834.

## 8123 C

# Inactivation of Histamine in Vivo.

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One of us with Gebauer-Fuelnegg[1] has previously reported on the appearance of a physiologically active substance in the blood and lymph of dogs during anaphylactic shock and on the basis of a pharmacological and chemical study[2] concluded that the active substance was apparently histamine. It was noted in this study that this active substance may be only temporarily detectable and it was concluded that it disappeared rapidly from the blood as it circulates. Various workers have noted the inactivation or detoxication of histamine in intact animals, organ perfusions, etc. (see Best and McHenry[3, 4] for discussion and literature). None of these studies, however, provide the necessary data to enable us to compare the fate of intravenously injected histamine with that liberated during shock. We were interested, therefore, in inducing shock of varying degrees of severity by intravenous injection of histamine at different dosage levels into dogs and of studying the blood and thoracic duct lymph for its presence and disappearance and of correlating these findings with our observations in anaphylactic shock.

Dogs were anesthetized with ether and sodium barbital and freshly prepared solutions of histamine acid phosphate (1-500) injected into the femoral vein. Samples of blood and thoracic duct lymph were collected at various time intervals thereafter. These samples were kept from clotting by means of heparin, the bloods in addition were centrifuged to obtain the plasma, and then assayed for their histamine activity by intravenous injection into cats (under ether or ether and barbital anesthesia and after the preliminary administration of atropine). The essential data of these experiments are outlined in Table I.

The degree of sensitivity of the cats used was always such that samples of blood or lymph recorded as negative contained a concentration of histamine less than 1-200,000. It is therefore apparent that injected histamine disappears very rapidly from the blood

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[1] Dragstedt, C. A., and Gebauer-Fuelnegg, E., *Am. J. Physiol.*, 1932, **102**, 512.

[2] Gebauer-Fuelnegg, E., and Dragstedt, C. A., *Am. J. Physiol.*, 1932, **102**, 520.

[3] Best, C. H., and McHenry, E. W., *J. Physiol.*, 1930, **70**, 349.

[4] Best, C. H., and McHenry, E. W., *Physiol. Rev.*, 1931, **11**, 371.

TABLE I.

Duration of Detectable Histamine in Blood Plasma and Thoracic Duct Lymph after Intravenous Injection of Histamine. (Dog.)

| | | | | Duration of detectable Histamine in blood plasma | | Duration of detectable Histamine in thoracic duct lymph | |
|---|---|---|---|---|---|---|---|
| Exp. No. | Histamine Ac. Phos. mg. per kilo | Calculated Initial conc. of H. in plasma | Degree of shock | Last positive sample, min. | First negative sample, min. | Last positive sample, min. | First negative sample, min. |
| A50 | 0.1 | 1-500 M | + | — | 1 | — | 0-10 |
| A168 | 0.5 | 1-100 M | ++ | 2 | 5 | | |
| A6 | 0.66 | | ++ | | | | 0-20 |
| 1 | 1.0 | 1-50 M | ++ | 3 | 10 | | 0-20 |
| A168 | 1.0 | ” | ++ | 2 | 10 | | |
| 5 | 1.0 | ” | ++ | | 5 | | 0-22 |
| 2 | 1.0 | ” | ++ | | | | 0-20 |
| A173 | 1.0 | ” | ++ | 1 | 5 | | |
| A134 | 1.5 | 1-37.5 M | ++ | | | 0-10 | 10-20 |
| A14 | 1.5 | ” | ++ | | | | 0-20 |
| 10 | 1.5 | ” | ++++(10′) | 10 | | | |
| 12 | 1.5 | ” | ++ | 10 | 20 | | |
| A18 | 1.6 | | ++ | | | | 0-20 |
| 4 | 2.0 | 1-25 M | ++++(7′) | | | | 0-7 |
| 3 | 2.0 | ” | ++ | | | | 0-20 |
| 6 | 2.0 | ” | ++ | 10 | 15 | 10-20 | 20-30 |
| 8 | 3.0 | 1-16.6 M | +++ | 15 | 20 | 15-20 | 20-28 |
| 7 | 3.0 | ” | +++ | 10 | 20 | 5-10 | 10-20 |
| 11 | 3.0 | ” | ++ | 30 | 40 | | |
| 9 | 4.0 | 1-12.5 M | +++ | 40 | 50 | 20-33 | 33-43 |
| 3 | 4.0 | ” | ++++(15′) | 15 | | 0-15 | |
| 5 | 4.0 | ” | ++++(7′) | 7 | | 0-7 | |

Explanatory: Degrees of shock: ++ = definite shock but recovery of blood pressure within 30 min.; +++ = severe shock, blood pressure still at shock level at 30 min.; ++++ = fatal shock. Time refers to minutes elapsed since injection of histamine.

stream. When a dose of 0.5 mg. per kilo is injected, which would give an approximate initial concentration in the plasma of 1 to 100,000 (assuming the plasma volume to be 1/20 body weight) histamine is detectable by this method of assay 2 minutes after the injection but not at 5 minutes. At 1.0 mg. per kilo it is also as a rule not detectable at 5 minutes. At 2.0 mg. per kilo, traces are still present for 10 minutes but usually gone by 15 or 20 minutes. At doses of 3.0 to 4.0 mg. per kilo (if not fatal sooner) traces are present for 30 to 40 minutes. We were never able to detect histamine in the thoracic duct lymph after doses of 1.0 mg. per kilo or less, occasionally after doses of 1.5 to 2.0 mg. per kilo, and regularly after doses of 3.0 to 4.0 mg. per kilo.

The similarity of these findings in the case of histamine with our experiences with the “anaphylatoxin” of anaphylactic shock is striking. In the great majority of the experiments on anaphylactic shock which were done early in our work, blood samples were frequently taken at what was assumed to be the "height" of shock, namely 5 to 15 minutes after the shocking dose of serum. Consequently we had many experiments of moderate degrees of shock in which the blood samples examined were negative, although the thoracic duct lymph samples were positive. These latter were, however, collected continuously from the onset of shock. In view of our findings on the rapidity of the disappearance of injected histamine we have repeated a number of experiments on anaphylactic shock and are now able to report that in anaphylactic shock of moderate degrees of severity, blood samples taken at one, 2 or 3 minutes after the shocking dose of serum are usually active, although later samples may be negative. It is also seen from this work that an occasional animal may die from a dose of histamine (1.5 mg. per kilo) which is insufficient to be regularly detected in the thoracic duct lymph. In our original report on anaphylactic shock it was noted that in 2 of 9 cases of fatal shock we failed to find any activity in the thoracic duct lymph. These findings with histamine therefore afford circumstantial evidence of the validity of the histamine theory of anaphylactic shock in the dog.

## 8124 P

## Muscle Atrophies. I. Water and Nitrogen Studies in Simple Disuse Atrophy.

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Histologic, chemical, and physiologic studies have been undertaken in various types of experimentally produced muscle atrophies in young *Macacus rhesus* monkeys. In a series of 6 animals, simple disuse atrophy was produced in the right leg of each animal by immobilizing it in a plaster cast. The left leg was used as the control in each case. The gastrocnemius and soleus muscles were completely removed by careful dissection from their proximal and distal attachments while the animals were under light ether anesthesia. The excised muscles (6 atrophied and 6 controls) were then weighed and split longitudinally, one-half being used in the preparation of histologic sections and the other used for chemical studies. The half used for chemical analyses was weighed immediately, sliced into several pieces, and desiccated over sulphuric acid under reduced vapor pressure (20-30 mm. Hg.) until a constant weight was obtained. Nitrogen was then determined quantitatively by the Kjeldahl method, duplicate determinations being made for each of the 12 specimens, and the protein content calculated.

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* This work was carried on with the assistance of a grant from the Council on Physical Therapy of the American Medical Association.

The loss in muscle weight varied from 4.9% in the one-week atrophy to 32.5% in the 10-week atrophy. Despite the decrease of muscle bulk in the atrophied muscle as compared with the respective controls, the relative proportions of water and protein remained substantially the same as in normal muscle. In the 10-week atrophy, for example, the variation in protein content between the control and the atrophied leg was but 0.3% and the difference in water content negligible.

Histologically the chief finding was a uniform shrinkage of the muscle cells. The muscle fibers were narrower but the characteristic appearance of the transverse striations remained unaltered. There was no increase in sarcolemmal or muscle nuclei nor in the connective tissue. The intramuscular blood vessels, nerves, and nerve endings were likewise unaltered. The significant change observed consisted of a diminution of the cross-section area. This change seemed to involve primarily the sarcoplasm, as evidenced by a more compact arrangement of the sarcostylic elements within the individual muscle cells. This was especially marked in cross-section in which Cohnheim's areas were no longer visible. The sarcostyles appeared distributed in a uniform manner throughout the muscle cells.

Electrical stimulation of the atrophied muscles with faradic and galvanic currents gave prompt responses, similar to those obtained in normal skeletal muscle.

### 8125 P

## Hypothalamus and Regulation of Body Temperature.*

S. W. RANSON AND W. R. INGRAM.

*From the Institute of Neurology, Northwestern University Medical School.*

Recent work[1] has shown that the hypothalamus plays an important part in the regulation of body temperature. In the course of a series of investigations[2] on the functions of the hypothalamus we have had occasion to make lesions in this part of the brain in the rhesus monkey *(Macaca mulatta)* and have kept records of the resultant changes in body temperature. Bilaterally symmetrical electrolytic lesions were made with the aid of the Horsley-Clarke stereotaxic instrument, used in the manner described elsewhere.[3] The lesions were relatively small and sharply localized. The electrode, 0.9 mm. in diameter, did very little damage on its way down through the cerebral hemisphere. In some of the animals, parts of the thalamus were involved due to the interruption of their blood supply by the more ventrally placed lesions, but these thalamic lesions were not, as a rule, extensive and varied in their location from animal to animal without any corresponding change in body temperature. Two animals, which retained normal thermal regulation, had as extensive damage in the thalamus as any of the others. Moreover, in 3 of the animals (MC 10, MC 25 and MC 26) with the most marked disturbance in temperature regulation the thalamus remained undamaged. The results to be described were, therefore, due to the lesions in the hypothalamus, the rest of the nervous system being essentially intact.

Nine of the monkeys lost the capacity to prevent the body temperature from falling below a normal level. These animals were kept for many days, in one case as long as 10 weeks, in a small room, the temperature of which was regulated at 86°F. Under these conditions these monkeys maintained a normal temperature

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* Aided by grants from the Rockefeller Foundation and from the Ella Sachs Plotz Foundation.

[1] Keller, A. D., and Hare, W. K., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1069; Bazett, H. C., Alpers, B. J., and Erb, W. H., *Arch. Neurol. and Psychiat.*, 1933, **30**, 728; Davison, C., and Selby, N. E., *Arch. Neurol. and Psychiat.*, 1935, **33**, 570.

[2] Ranson, S. W., Trans. of Col. of Phys. of Philadelphia, 1934, Series IV, 2, 222; Ingram, W. R., Fisher, C., and Ranson, S. W., *Arch. Int. Med.*, in press.

[3] Ranson, S. W., *Psychiat. en neurol. bl.* (Feestb. C. U. Ariëns Kappers), 1934, **38**, 534.

except that sometimes during the first few days it would be slightly subnormal. If in the days immediately following the operation such an animal was removed for a few hours from the warm room to one ranging from 70° to 75°, the rectal temperature would fall rather rapidly. Gradually in the course of days or weeks these animals regained at least a fair degree of thermal control.

Seven other monkeys exhibited a fairly sharp post-operative rise in rectal temperature as the effect of the nembutal anesthesia wore off, reaching 105° to 106.5°F. within from 7 to 11 hours after the administration of the anesthetic and 5 to 9 hours after the completion of the operation. Most of these rises occurred at ordinary room temperatures but the first monkey in which this sharp rise was noted had been placed in the warm room (86°). None of these 7 animals developed a fatal hyperpyrexia and the temperature dropped to normal or to a point only slightly above normal within a day or 2 after the operation. It is interesting to speculate on the relation of this post-operative rise to the hyperpyrexia sometimes seen in patients after operations involving the hypothalamus.

Microscopical examination of the brains revealed that those monkeys which had shown a post-operative rise all had lesions involving bilaterally the rostral part of the lateral hypothalamus except one in which the damage was more medially placed and involved the lateral hypothalamus on only one side. In no case did the lesions extend caudally far enough to damage the region lateral to the mammillary nuclei. The lesions involved the fornix and the lateral part of the hypothalamus, extending rostrocaudally from the level of the optic chiasma to a point near the level of the rostral end of the mammillary nuclei. On the other hand, those monkeys which became cold when removed from the warm room all had bilaterally symmetrical lesions destroying the posterior part of the lateral hypothalamus, *i. e.,* the region lateral to the mammillary nuclei. The amount of involvement of the mammillary nuclei themselves varied greatly and did not seem to be an essential factor. The contrast between the lesions seen in these 2 groups of monkeys is fully as striking as the differences in their thermal reactions. Two animals may be selected as illustrative of these 2 groups. Before referring to them it may be well to mention that the normal rectal temperature of the monkey is about 101°F.

Monkey MC 10 developed a rectal temperature of 106.5° in the warm room (86°) seven hours after the administration of the anesthetic and about 5 hours after the completion of the operation. It was then removed to a cooler place and kept at ordinary room temperature. The next morning the rectal temperature was 105.2°. The morning of the following day it was 103.3°. Subsequently a normal temperature was maintained. The lesions were typical of those described for the group of monkeys exhibiting a post-operative rise in temperature and the thalamus was uninjured.

Monkey MC 25 ran a subnormal temperature ranging from 97.6° to 99.9° during the first week in the warm room; and on the third day, when it was removed to a room at 70.8°, its rectal temperature dropped from 98.8° to 95.3° in one hour. On the thirty-eighth day its rectal temperature on removal from the warm room was 100.0° and it fell to 98.5° in 7 hours in a room the temperature of which varied from 70°-72°. The lesions destroyed the lateral hypothalamic area on both sides from the level of the optic chiasma to the caudal border of the mammillary bodies but did not involve the thalamus.

It may be concluded that bilateral destruction of the caudal part of the lateral hypothalamus causes a prolonged loss of the capacity to keep the body temperature up to normal and that lesions in the rostral part of the lateral hypothalamus may cause a temporary rise in body temperature if the caudal part remains intact.

## 8126 C

## Oxidation-Reduction Potentials of Some Non-Sporulating Obligate Anaerobes.

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Oxidation-reduction potential studies have been made on cultures of many aerobic and some sporulating anaerobic bacteria, but to our knowledge none has been made on the non-sporulating obligate anaerobes. The potentials produced by the latter group of organisms are of particular interest in connection with current theories as to the relation of such potentials to the growth of obligate anaerobes. A certain degree of negativity was thought to be necessary before the obligate anaerobes are able to initiate their growth processes. Experimental evidence of a positive limit of oxidation-reduction potential required for the germination of spores of *Cl. tetani* has been presented by Fildes[1] and by Knight and Fildes.[2]

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[1] Fildes, *Brit. J. Exp. Path.*, 1929, **10**, 151.

[2] Knight and Fildes, *Biochem. J.*, 1930, **24**, 1496.

This positive limit was found to lie in the vicinity of $E_h$ 0 at pH's close to neutrality. The potentials produced in cultures of the sporulating anaerobes are considerably more negative than this positive limit, usually being about 350 millivolts negative at reactions at or near neutrality.[3] In unpublished experiments one of us (W.B.) has found that several strains of *Cl. botulinum* produce potentials around 400 millivolts negative. That anaerobiosis is a matter of oxidation-reduction potential rather than simple absence of oxygen is a theory that has been generally accepted,[4] although there has been some dissent.[5]

The technique used in the present experiment has been previously described.[6] The organisms were grown in a cystine glucose beef infusion broth, the pH varying between 6.4 and 6.9. Anaerobic conditions were produced and maintained by constant bubbling of oxygen-free nitrogen through the medium. Very heavy inoculums were used, usually 1 to 2 cc. of young cultures. Under these conditions we had no difficulty in growing the organisms.

With 2 exceptions, the strains used were isolated from the colons of patients suffering with ulcerative colitis. Strain 39 was isolated from the ulcerated colon of a monkey dying from bacillary dysentery (Flexner) and strain M1 was isolated from the colon of a healthy monkey. Descriptions of these organisms are given elsewhere.[7, 8]

The following strains were used:

| *Strain No.* | *Morphology* |
|---|---|
| 26 | Gram variable streptococcus |
| 39 | Pleomorphic Gram negative rods and filaments |
| 40 | Fusiform-like Gram negative rods with occasional filaments |
| 45 | Pleomorphic Gram negative rods and filaments. Resembles strain 11[7] |
| 47 | Pleomorphic Gram negative rods and filaments. Resembles strain 39[7] |
| M1 | Small Gram negative rods (Bacteroides)[8] |

The results of these experiments appear in Fig. 1. These cultures were known to lyse, some more readily than others. That such lysis may be accompanied by a positive drift in potential has been shown by Hewitt[9] with a number of species of bacteria. This

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[3] Plotz, H., and Geloso, J., *Ann. Inst. Past.*, 1930, **45**, 613.

[4] Hewitt, L. F., Oxidation-Reduction Potentials in Bacteriology and Biochemistry, London County Council, 2nd ed., 1934.

[5] Knaysi, G., and Dutky, S. R., *J. Bact.*, 1934, **27**, 109.

[6] Burrows, W., and Jordan, E. O., *J. Inf. Dis.*, in press.

[7] Dack, G. M., Heinz, T., and Dragstedt, Lester, *Arch. Surgery*, in press.

[8] Barringer, Sarah J., and Dack, G. M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1125.

[9] Hewitt, L. F., *Biochem. J.*, 1931, **25**, 1452.

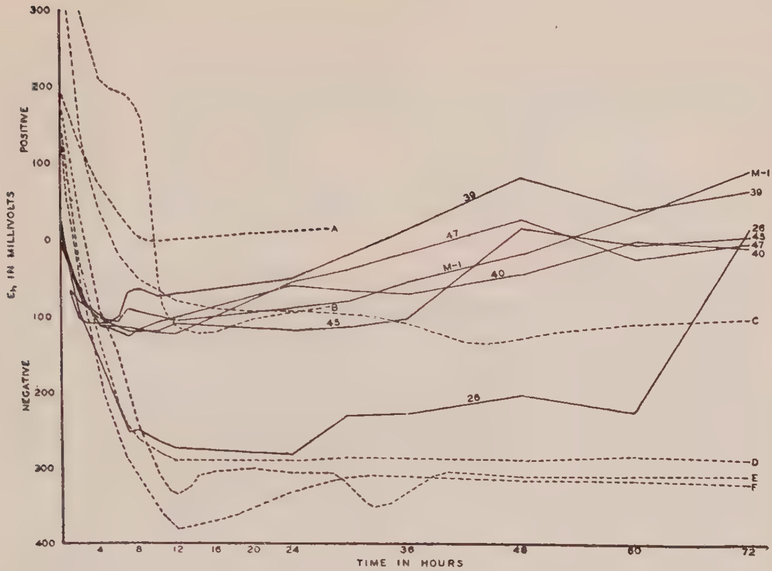

Fig. 1.

Time-Potential Curves Showing the Relations Between the Non-Sporulating Obligate Anaerobes and Other Miscellaneous Bacteria.

The solid lines represent the non-sporulating obligate anaerobes. The strain numbers are appended in each case. The dotted lines are made up from data of other workers on miscellaneous species of bacteria. A—sealed culture of a hemolytic streptococcus in cystine broth[10]; B—aerobic culture of a hemolytic streptococcus in cystine broth;[10]; C—a staphylococcus;[11] D—Cl. welchii;[3] E—Cl. botulinum;[3] F—Cl. sporogenes.[3].

finding is confirmed in our experiments; in each case the rise in potential appears when lysis begins to take place. The point we wish to emphasize, however, is that these organisms do not produce extremely negative potentials in their cultures as might be expected. Certainly their chemistry appears to be more nearly like that of the aerobes and facultative anaerobes than that of the sporulating obligate anaerobes. It is apparent, therefore, that all obligate anaerobes do not produce very negative potentials nor are such potentials necessary to their growth processes. These experiments do not, however, provide evidence contrary to the theory that anaerobiosis is a matter of oxidation-reduction potential, since the potentials produced by these organisms are well within the positive limits found by Knight and Fildes.[2]

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[10] Hewitt, L. F., *Biochem. J.*, 1930, **24**, 512.

[11] Hewitt, L. F., *Biochem. J.*, 1931, **25**, 2068.

### 8127 P

## Relation of Olfactory Tracts to Intravenous Route of Infection in Experimental Poliomyelitis.

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Epidemiological and experimental studies indicate that human infection with the virus of poliomyelitis occurs chiefly through the nasopharynx. Flexner and Lewis[1] first showed that poliomyelitis could be produced in monkeys by intranasal instillation of the virus. Flexner and Clark[2] found the olfactory lobes infective 48 hours after such inoculation and Fairbrother and Hurst[3] and Faber[4] have been able to demonstrate the probable route of passage of the virus from the nasopharyngeal mucosa to the spinal cord. The concept that the upper respiratory tract is the portal of entrance for the virus is supported by the detection of the virus in washings or tissues of the nasopharynx,[5] both in the experimental and in the human disease, as well as in healthy contacts[6] and carriers.[7] Further evidence has been adduced by Brodie and Elvidge[8] and by Schultz and Gebhardt,[9] who showed that section of the olfactory tracts in monkeys prevents infection by the nasal route. We have confirmed and extended these observations.

The olfactory tracts of 5 *Macacus rhesus* monkeys were sectioned by a frontal approach. Five months later these animals were given 2.5 cc. 10% PMV virus intranasally on each of 3 days. The nose was irrigated with phosphate buffer solution of pH 5.0 prior to each inoculation of virus. None of these 5 monkeys had any signs of infection, whereas all of 9 animals with unsectioned tracts used as controls in various experiments and treated similarly, died of poliomyelitis.

The ineffectiveness of the intravenous route of infection in experimental poliomyelitis, unless massive doses of virus are used, is well known and generally attributed to the inability of the virus to reach the central nervous system. We thought it of interest to determine the effect of olfactory nerve section on the production of poliomyelitis after intravenous injection of virus since, in a number of virus diseases, the causative agent is known to be excreted into the nasopharynx (*e. g.*, Shope[10] has found that the virus of pseudorabies, after intramuscular injection in swine, appears in the nasal secretions at the onset of symptoms). Accordingly, 5 monkeys with sectioned olfactory tracts and 5 intact control animals were given a daily intravenous injection of 10 cc. 10% virus on 3 successive days. None of the former animals showed any evidence of infection whereas 4 of the 5 controls succumbed to poliomyelitis. These clear-cut results lead us to believe that the virus under these conditions is excreted from the blood stream onto the nasal mucosa, where it enters the endings of the olfactory nerves and migrates to the central nervous system. If such excretion occurs, the virus should be recoverable from the nasal washing. Four immune and 6 normal monkeys were given 2 daily intravenous injections of 10 cc. 10% virus. None of the immunes became infected; 5 of the 6 controls died of poliomyelitis. On the 2nd, 4th, and 6th days of the experiment the nasopharynx of each monkey was washed out with about 20 cc. sterile distilled water. The washings of each day were pooled in 3 groups—the immunes constituted 1 group, the normals 2 groups of 3 animals each. After pooling, the washings were passed through a Berkefeld N filter, concentrated to from 2 to 6 cc. by boiling *in vacuo* at 37°C., and injected intracerebrally into test monkeys in 2 cc. amounts. The results were negative except in one instance, when the test animals receiving the pooled washings obtained on the fourth day from one group of normal monkeys showed weakness and a rise in temperature. It died on the 12th day after inoculation and microscopic examination of the cord revealed typical poliomyelitic lesions. In several other instances there also occurred a rise in temperature, but the microscopic findings in the spinal cords of these animals (sacrificed) were not histologically diagnostic of poliomyelitis. When the difficulties inherent in detecting poliomyelitis virus in nasal washings are considered, the one positive result is of significance.

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[1] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, **54**, 1140.

[2] Flexner, S., and Clark, P. F., PROC. SOC. EXP. BIOL. AND MED., 1912, **10**, 1.

[3] Fairbrother, R. W., and Hurst, E. W., *J. Path. and Bact.*, 1930, **33**, 1133.

[4] Faber, H. K., *Medicine*, 1933, **12**, 83.

[5] ''Poliomyelitis,'' International Committee, Williams and Wilkins, Baltimore, 1932.

[6] Kramer, S. D., *J. Am. Med. Assn.*, 1932, **99**, 1048.

[7] Kramer, S. D., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1165.

[8] Brodie, M., and Elvidge, A., *Science*, 1934, **79**, 235.

[9] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728.

*Summary*. Section of the olfactory tracts of 5 monkeys prevented infection with poliomyelitis virus when administered intranasally. Later, these animals could not be infected by the intravenous route.

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[10] Shope, R. E., *Science*, 1934, **80**, 102.

We believe this is explicable on the basis that the virus, which has been detected in the nasopharynx by ourselves and others, did not reach the central nervous system after excretion onto the nasal mucosa, because of the break in the olfactory pathway.

## 8128 P

## Hyperthyroidism and Brain Oxidations.

R. A. COHEN. (Introduced by R. W. Gerard.)

*From the Department of Physiology, University of Chicago.*

Many workers have shown that general tissue metabolism is increased in hyperthyroid animals; few have attempted an analysis of the specific cell enzymes involved. Dye and his coworkers[1] reported such studies on hypothyroid dogs, and McEachern[2] on thyroxinized rats (liver, kidney and muscle). The present experiments on hyperthyroid rat brain, directed to this end, were begun before the appearance of McEachern's paper.

Male white rats (160 to 190 gm.) were used. Experimental animals were fed the same stock diet as controls, plus 0.6 gm. desiccated thyroid daily for 16-19 days. A brei of finely minced whole brain was suspended in phosphate Ringer and its oxygen consumption determined at 37°C. in Warburg manometers.

Typical substrates were used alone and with dyes and/or inhibitors. Because of great variations in the early readings (probably determined by the amount of substrate initially present) the tissue was allowed to respire for 2 hours, at which time remarkably constant readings were obtained. The substrates were then tipped in, and the oxygen consumption for the next 90 minutes determined. With few exceptions each figure given is based on 6-10 experiments.

Results. (1) The $Qo_2$ of hyperthyroid brain (H) is initially 20% greater than that of normal brain (N), becomes equal to it in 2 hours, and is 10% lower in 4½ hours (due to substrate exhaustion).

(2) The $Qo_2$ of H is increased approximately 4 times as much

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[1] Dye, J. A., *Am. J. Physiol.*, 1933, **105**, 518; Dye, J. A., and Waggener, R. A., *Ibid.*, 1928, **85**, 1; Dye, J. A., and Maughan, G. H., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 439.

[2] McEachern, D., *Bull. Johns Hopkins Hosp.*, 1935, **56**, 145.

TABLE I.

$Q_{O_2}$ of brei plus inhibitor and substrate is expressed in: I as per cent of brei + substrate without inhibitor; II as per cent of brei + inhibitor without substrate.

| Added | | M/1000 NaCN | | | M/1000 NaCN + .02% Cresyl blue | | | M/100 Iodoacetate | | | M/100 Malonate | | | 0.1% Barbital | | | 0.1% Urethane | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Substrate | | N | H | H/N | N | H | H/N | N | H | H/N | N | H | H/N | N | H | H/N | N | H | H/N |
| None | I | 20 | 18 | 0.9 | 69 | 63 | 0.9 | 13 | 13 | 1.0 | 96 | 96 | 1.0 | 94 | 93 | 1.0 | 97 | 101 | 1.0 |
| | II | | | | 223 | 255 | 1.1 | | | | | | | | | | | | |
| Glucose | I | 23 | 28 | 1.2 | 28 | 28 | 1.0 | 34 | 34 | 1.0 | | | | 103 | 102 | 1.0 | 100 | 100 | 1.0 |
| | II | 183 | 275 | 1.5 | 224 | 275 | 1.2 | 365 | 430 | 1.2 | | | | 122 | 183 | 1.5 | 121 | 181 | 1.5 |
| Lactate | I | | | | | | | 12 | 22 | 1.8 | | | | 93 | 89 | 0.9 | 152 | 152 | 1.0 |
| | II | | | | | | | 154 | 297 | 1.9 | | | | 164 | 179 | 1.1 | 130 | 238 | 1.8 |
| Succinate | I | 12 | 11 | 0.9 | 83 | 75 | 0.9 | 21 | 22 | 1.0 | 31 | 25 | 0.8 | 80 | 80 | 1.0 | 100 | 100 | 1.0 |
| | II | 187 | 293 | 1.6 | 1240 | 1900 | 1.5 | 423 | 755 | 1.8 | 134 | 139 | 1.0 | 125 | 348 | 2.8 | 194 | 485 | 2.5 |
| P-phenylene-diamine | I | 22 | 22 | 1.0 | 27 | 28 | 1.1 | 90 | 96 | 1.1 | 106 | 98 | 0.9 | 99 | 103 | 1.0 | 100 | 100 | 1.0 |
| | II | 490 | 560 | 1.2 | 505 | 700 | 1.4 | 2230 | 3150 | 1.4 | 360 | 424 | 1.2 | 347 | 440 | 1.3 | 350 | 435 | 1.4 |

as that of N on adding substrates (S) which are probably involved in carbohydrate oxidation and glycolysis, even though the absolute increases for individual substrates varied widely. Percent increase $= \frac{N+S}{N} \times 100$ and $\frac{H+S}{H} \times 100$ for each substrate: glycogen = 3, 12; glucose = 18, 81; fructose = 72, 190; glycerophosphate = 22, 74; lactate = 19, 86; succinate = 92, 385. For p-phenylenediamine the increase in H is 35% greater than N (250, 337); while the increase for other substrates is essentially the same for N and H; (pyruvate = 68, 72; galactose = 32, 33; glycine = 11, 10; methyl glyoxal = 18, 16).

(3) Methylene blue (0.01%) and cresyl blue (0.02%) affect H and N alike, leading to an early increase (30 and 25% respectively in 15 minutes) and a later decrease of $Q_{O_2}$ (equal at 30 minutes, 20 and 30% in 90 minutes).

(4) See Table I. Inhibitors decrease the respiration of brei and substrate by the same percentage in both H and N. On the other hand, the percentage increase of $Q_{O_2}$ produced by adding substrates to inhibited brei is always distinctly higher in H than in N. These are consequences of the fact that with inhibitors alone H and N are alike in absolute values, while with substrates alone H is more than N.

*Conclusions.* These data suggest that the increased $Q_{O_2}$ of hyperthyroid brain is especially associated with carbohydrate oxidations. The results with inhibitors further indicate that this greater respiration depends on proportional increases of both dehydrases and oxidases in the normal cell; but stronger concentrations of narcotics must be used to clinch this point. These results do not exclude Haffner's,[3] or allied hypotheses, which regard an increased glycolysis as the essential metabolic effect of hyperthyroidism and the increased $Q_{O_2}$ as a secondary consequence.

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[3] Haffner, F., *Klin. Wochnschr.*, 1927, **6**, 1932.

## 8129 P

# Metabolism of Tissues in Cerebrospinal Fluid and Other Media.

J. E. DAVIS AND A. B. HASTINGS.

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Artificial media, approximating the normal fluid environment of cells, are widely used for the study of the metabolism of excised tissues. In the differential method of Warburg[1] it is possible to employ a solution which has the physiologically normal calcium ion concentration of 1 millimol per liter at pH 7.4. The composition of the solution which we refer to as our "standard" solution of reference is given in Table I.

TABLE I.
Metabolism of Tissues in Various Media.

Composition of solutions:

I. Standard solution. NaCl, 114 mM/L; $NaHCO_3$, 25mM/L; KCl, 6 mM/L; $CaCl_2$, 1.4 mM/L; $Na_3$Citrate, 0.7 mM/L; $Na_2HPO_4$-$NaH_2PO_4$, 1.0 mM/L; Glucose, 0.2%. ($Ca^{++}$ = 1.0 mM/L, pH = 7.4.)

II. Solution I plus sodium indigo disulfonate, 0.1 mM/L, and without $Na_3$Citrate.

| | | | Metabolism | | |
|---|---|---|---|---|---|
| Tissue | Medium | No. of Experiments | $Q_{O_2}$ cmm./mg./hr. | % Change | $Q^{O_2}_{CO_2}$ cmm./mg./hr. |
| Rat liver | Solution I | 5 | 10.2 | — | 0.0 |
| " " | Cerebrospinal Fluid | 5 | 14.9 | +46 | 0.0 |
| " " | Solution II | 5 | 17.7 | +74 | 0.8 |
| Mouse liver | Solution I | 5 | 12.4 | — | 2.0 |
| " " | Cerebrospinal Fluid | 5 | 14.4 | +16 | 1.0 |
| " " | Solution II | 5 | 17.3 | +40 | 2.8 |

Serum has been used successfully by Warburg[2] as a medium for the study of the metabolism of excised tissue. The complication in technique which arises from the buffer action of the serum proteins has been one of the drawbacks to its widespread use in the differential method, however. On the other hand, cerebrospinal fluid has all the biological advantages of serum and, in addition is protein-free. This permits its direct use as a fluid medium for excised tissues. Through the cooperation of the Department of Neurology, we have had an opportunity to compare the metabolism of tissues in cerebrospinal fluid with that in our standard salt solution medium. The results are given in Table I. In every instance the oxygen consumption has been *higher,* and the aerobic glycolysis has, when present, been *lower,* in the cerebrospinal fluid than in the standard salt solution.

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[1] Warburg, O., *Biochem. Z.*, 1924, **152**, 51.

[2] Warburg, O., *Biochem. Z.*, 1925, **164**, 481.

The reason why the cerebrospinal fluid should thus appear to be a superior medium for the metabolism of mouse liver is not known, although we are not inclined to attribute its superiority to differences in concentrations of inorganic ions.

An example of one way in which the metabolism of tissues may be markedly increased by the presence of organic substances—in this instance a reversibly oxidizable dye, indigo disulfonate—is also shown in the table.

## 8130 C

## Blood Purine Derivatives in Migraine.

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In an effort to uncover any possible abnormality in purine metabolism, the blood of 12 patients who suffer from migraine headaches was analyzed for adenine nucleotide nitrogen, purine nitrogen, and uric acid.

Methods for the determination of these substances in blood have, in the past, been unreliable. So-called normal values varied considerably according to the method used. Although the method employed in this study appears to be a distinct improvement on those formerly used, its reliability in the hands of different investigators remains to be established. Since its introduction no published reports of its use have appeared. Because the values obtained by this method cannot be compared with those obtained by other methods it was necessary to study the blood of normal subjects for comparative purposes.

The blood was obtained from 3 of the migraine patients during attacks. From the remaining 9 it was obtained during the interval between attacks. The determinations for adenine nucleotide nitrogen, and purine nitrogen were made according to the methods of Kerr and Blish[1]; those for uric acid according to Benedict's method.[2] All determinations were made in duplicate on fasting blood.

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[1] Kerr and Blish, *J. B. C.*, 1932, **98**, 193.

[2] Benedict, *J. B. C.*, 1931, **92**, 161.

TABLE I.

| Patient | Adenine Nucleotide N | Purine N | Uric Acid |
|---|---|---|---|
| | Migraine Patients. | | |
| 1 | 12.1 | — | 2.9 |
| 2 | — | 4.1 | — |
| 3 | 12.1 | 3.3 | —* |
| 4 | 11.8 | 3.6 | 2.8 |
| 5 | 11.6 | 5.3 | 2.3 |
| 6 | 14.5 | — | 3.6 |
| 7 | 9.8 | 4.3 | — |
| 8 | 9.6 | — | 2.8* |
| 9 | 12.1 | 4.3 | 3.1 |
| 10 | 13.0 | 4.0 | 2.8 |
| 11 | 11.5 | 3.5 | 3.2* |
| 12 | 12.1 | 6.6 | — |
| | Normal Patients. | | |
| Subject | | | |
| 1N | 12.1 | 4.3 | |
| 2N | 11.7 | 5.0 | |
| 3N | 10.4 | 4.8 | |

*Blood drawn during attack of migraine.

The results are listed in Table I. In the migraine series the average values for adenine nucleotide nitrogen and purine nitrogen were 11.8 and 3.9 mg. per 100 cc. respectively. In the normal series the average values were 11.3 mg. per 100 cc. for adenine nucleotide nitrogen and 4.7 mg. per 100 cc. for purine nitrogen.

Although normal values cannot be established on the basis of 3 cases, the absence of variation between patients and controls indicates no departure from the normal in migraine. Since the entire group of 15 patients and controls show only slight variation in nucleotide and purine nitrogen, this study may be considered to indicate the normal fasting values for these constituents of the blood.

These studies are not suggestive of any abnormality of purine metabolism in migraine.

## 8131 P

# Progestin in Control of Human Uterine Contractions. Significance in Prevention of Habitual and Threatened Spontaneous Abortion.

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The hormone progestin, extracted from the corpus luteum, has been used experimentally in the lower animals to inhibit uterine contractions. Its use clinically has been reported in women who were aborting and in those with a history of habitual abortion. The apparent reason for its effectiveness in such cases is its antagonistic action to the oxytoxic action of pituitrin. We reported[1] a series of 19 cases, some of which were threatened abortions and some habitual abortions. These women had lost 69% of their babies in 38 previous pregnancies under ordinary management, while the same women lost only 26% of their babies when treated with progestin, but without morphine or other sedative.

To test out the effect of progestin on the contractions of the human uterus, a metreurynter bag was introduced into the uterus on the seventh day post partum and inflated sufficiently to stimulate weak uterine contractions, according to the method of Moir.[2] The bag was connected to a recording tambour by a long rubber tubing. The writing lever traced variations in uterine contractions on a slowly revolving drum. The effect of injections of estrin, progestin and pituitrin were studied.

The insertion of a bag into the human post partum uterus at the seventh day is very easily done without anesthesia, since the cervix is sufficiently patulous to admit the folded bag and yet has contracted sufficiently to prevent the bag from being extruded into the vagina after moderate inflation. At the seventh day post partum, sufficient involutionary changes have taken place to obliterate the lower uterine segment, hence we considered that the contractions measured were those of the upper uterine segment. Ivy, Hartman and Koff,[3] Adair and Davis,[4] and others have shown that the lower uterine segment has no contractile power. Young and older primiparae and multiparae were used.

In general, it may be said that there are many differences in the reactions of normal uteri which must be taken into account when evaluating the effect on uterine contractions of the injection of any stimulating or sedative substance.

The patients complained only slightly about the injections or the length of the experiment. There was no fever or untoward reaction clinically in any of these patients and they were discharged from the hospital in the usual number of days.

The normal response of a hollow organ to the presence of a foreign body in its lumen is peristaltic contraction to expel the object. Peristalsis of the gastrointestinal tract and painful uterine contractions in women who pass endometrial tissue or clots during menstruation, are examples of this action.

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[1] Krohn, Leon, Falls, F. H., Lackner, Julius E., *Am. J. Obs. and Gyn.*, 1935, **29**, 198.

[2] Moir, C., *Brit. Med. J.*, 1932, **1**, 1022.

[3] Ivy, A. C., Hartman, C. G., and Koff, A., *Am. J. Obs. and Gyn.*, 1931, **22**, 388.

[4] Adair, Fred L., and Davis, M. E., *Trans. of Am. Assn. Obs. and Gyn. and Abd. Surgeons*, 1933, **46**, 168.

The growing fetus and placenta must therefore stimulate the uterus to contract and expel its contents. That this does not occur in the majority of cases until the ninth month of pregnancy must be due to (a) deficient response of the uterus to the physiological stimulus, (b) under-production of the stimulating principles, or (c) to the action of some antagonistic substance which nullifies the action of the stimulating substance.

It has been conceded by most observers that the substance which causes uterine contractions is pituitrin (posterior lobe extract), or some closely allied substance. Its physiological effect, both in initiating and re-enforcing uterine contractions is well known. Hisaw[5] has demonstrated that progestin inhibits uterine contractions in the experimental animal. Morell[6] showed that the injection of progestin nullifies the effect of pituitrin. Corner and Allen[7] conclusively proved that the lutein hormone is essential to prevent abortion of early pregnancy. Miklos[8] revealed that progestin in excess prolongs gestation.

Oestrin on the other hand, according to Brouha and Simonnet,[9] sensitizes the uterus to pituitrin, acting in this way as an antagonist to progestin. Hirst,[10] Hofbauer,[11] Halban,[12] Antecki and Zwolinski,[13] Wolfsohn,[14] Weinzierl,[15] *et al.* have used various preparations of the extract of corpus luteum in the treatment of threatened or habitual abortion in women, but none of these have used a standardized dosage of progestin.

In our experimental work we used one rabbit unit ampoules of progestin carefully standardized. This inhibited uterine contractions and prevented the oxytoxic action of 1 cc. of pituitrin given intramuscularly to women in the seventh day of the puerperium. This inhibiting action occurs whether progestin is given before the injection of pituitrin or after such injection has started the uterine contractions. Under the latter condition, the inhibiting effect becomes apparent in a few minutes and lasts for at least 2 hours.

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[5] Hisaw, F. L., with Fevold, H. L., and Meyer, R. K., *Physiol. Zool.*, 1930, **3**, 135.

[6] Morell, with Mazer, Charles, and Goldstein, Leopold, *Clinical Endocrinology of the Female*, W. B. Saunders Company, 1932, 62.

[7] Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, **88**, 326.

[8] Miklos, L., *Z. f. Gynäk.*, 1930, **54**, 1755.

[9] Brouha, L., and Simonnet, H., *Compt. rend. Soc. de biol.*, 1927, **96**, 96.

[10] Hirst, J. C., *A Manual of Gynecology*, Philadelphia, 1925, W. B. Saunders Co.

[11] Hofbauer, J., *Zentralbl. f. Gynäk.*, 1920, **44**, 777.

[12] Halban, J., *München. med. Wchnschr.*, 1921, **68**, 1314.

[13] Antecki, S., and Zwolinski, T., *Polska Gazeta Lekarska*, 1928, **46**, 845.

[14] Wolfsohn, H., *Med. Welt.*, 1932, **6**, 1616.

[15] Weinzierl, E., *Med. Klin.*, 1933, **29**, 563.

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## 8132 P

## Presence of Vitamin C in Saliva.

O. H. STUTEVILLE. (Introduced by C. J. Farmer.)

*From the Department of Chemistry, Northwestern University Medical School, Chicago.*

The presence of Vitamin C has been demonstrated in various body tissues: adrenal,[1] pituitary,[2] ovary,[3] and tumors;[4] also the following fluids: blood serum,[5] cerebral spinal fluid,[5] aqueous humor,[5] and urine.[5, 6]

Van Eekelen, *et al.*, reported biological tests of Vitamin C in urine.[7] Johnson and Zilva fed urine to guinea pigs and found the biological value to equal the titrated value.[8]

Saliva was secreted by chewing paraffin and 7.5 cc. of saliva was acidified with 2.5 cc. of 20% trichloracetic acid, then filtered. Two cc. of the filtrate was titrated with 2-6-dichlorophenolindophenol which was prepared by dissolving 10 mg. of the dye in 50 cc. of water. This was then standardized against an ascorbic acid (Cebione, Merck) solution, which had been previously standardized against a standard iodine solution. The estimation was made by titrating the dye solution into 2 cc. of filtrate until a definite pale pink color was obtained.

KSCN was found not to interfere with the titration.

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[1] Harris and Ray, *Biochem.*, 1933, **27**, 303

[2] Gough and Zilva, *Biochem.*, 1933, **27**, 1279.

[3] Same as No. 2.

[4] Boyland, *Biochem.*, 1933, **27**, 802.

[5] Van Eekelen, M., Emmerie, A., Josephy, B., Wolff, L. K., *Klin. Wnschr.*, 1934, **13**, 564.

[6] Harris, Ray, and Ward, *Biochem.*, 1933, **27**, 2011.

[7] Van Eekelen, M., *et al.*, *Tagung des Niederlandischer V. reins fur Physiologie und Pharmakologie*, Dec. 18, 1933.

[8] Johnson and Zilva, *Biochem.*, 1934, **28**, 1393.

To further establish the presence of Vitamin C in saliva, a guinea pig which had been depleted for other purposes and was rapidly declining in weight was placed on a supplement of 4 cc. of saliva daily for 14 days, and later on 8 cc. for 11 days. The quantity of saliva fed did not cure the scurvy, but it prolonged the life of the animal and maintained the body weight above that of the initial value. The amount of Vitamin C given daily in 4 cc. of saliva, estimated by titration, averaged 0.010 mg.; the quantity being doubled with a supplement of 8 cc.

# Iowa Section

*State University of Iowa, May 17, 1935.*

## 8133 P

### Factors Involved in Lactation and Rearing of Young.

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The work reported in this paper is a continuation of studies by Guest, Nelson, Parks, and Fulmer.[1] These investigators demonstrated that rats grow at the normal rate with various single foods as the only sources of vitamin B. However, although reproduction was approximately normal on these diets, there was a high mortality of the young. Furthermore, a large number of females died during pregnancy or parturition on these diets, the mortality varying with the nature of the seed used as a source of vitamin B. The present paper reports additional data regarding the failure of lactation on certain synthetic diets.

Oatmeal, yellow corn, white corn, and barley were each fed at a level of 60% as sole sources of vitamins B and G; likewise, wheat was fed at a 30% level as a source of these vitamins, and rice polishings, wheat germ, rice bran, and corn germ were each incorporated in the rations at levels of 10% as the only sources of vitamins B and G. The synthetic ration was composed of casein 18%, salt mixture 3.7%, butterfat 4.0%, cod liver oil 1.0%, varying amounts of the different individual foods, and dextrin to 100%. The different rations were fed to pregnant females transferred from the growing ration to the experimental diets at the time of parturition. The data from these experiments show that the mortality of the young was in practically all cases very high. Only in a few instances was the weaning weight normal; and it was also found that a high weaning weight is not necessarily accompanied by a low mortality.

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[1] Guest, A. E., Nelson, V. E., Parks, T. B., and Fulmer, E. I., *Am. J. Physiol.*, 1926, **76**, 339.

Another series of experiments was performed to determine whether the foods above were deficient in vitamin B or vitamin G as far as lactation is concerned. To the diets in the first series of experiments was added 10% of autoclaved yeast as a source of vitamin G. The autoclaved yeast was prepared by moistening Fleischmann's dried yeast with distilled water—so as to form a thick paste—and autoclaving for 5 hours at 15 lb. pressure; it was then dried at 80°C. The autoclaved yeast markedly reduced the mortality of the young and enhanced the weaning weight of the young from the mothers on the various seeds and products from seeds tested. Corn germ was an exception to this general statement, since in this case 10% of autoclaved yeast reduced the mortality only moderately and had no effect on the weight of the young at the time of weaning. Since the autoclaved yeast contained no vitamin B, it is evident that the factor or factors responsible for this favorable effect reside in the G fraction of the yeast.

A preparation of vitamin B adsorbed on Fuller's earth was prepared, and a preparation of vitamin G from hog liver was made; the effect of these preparations on lactation was studied when added to rations containing 60% yellow corn or 60% wheat as the only sources of B and G. The mortality of the young was not reduced by the addition of 0.318 gm. of the vitamin B fraction daily to the yellow corn ration, but it was reduced markedly by the daily addition of 1.66 gm. of the vitamin G preparation to the yellow corn ration; furthermore, the weight of the young at the time of weaning was markedly increased by the latter addition. Similar results were obtained with the 60% wheat rations. Daily addition of 0.318 gm. of vitamin B preparation to the 60% wheat ration had only a slight effect on mortality and no effect on the weight of the young when weaned. However, the addition of 1.66 gm. of the vitamin G preparation per day to the 60% wheat ration reduced mortality of the young to zero and increased the weaning weight by 90%.

Experiments were then initiated to ascertain whether the 2 preparations of vitamins B and G would suffice for normal growth, reproduction, and lactation as the sole sources of these 2 vitamins. The basal ration had the same composition as the one employed for the previous experiments, except it contained 1.0% of wheat germ oil as a source of vitamin E. The preparations of vitamins B and G served as the only source of these vitamins in the diet. The rats were taken from the growing ration at the time of weaning and placed on the experimental diets. The mortality of the young on most of these rations was very high. On the lower levels of the vitamin B and vitamin G preparations the weaning weights were low; whereas, on the higher levels the weights of the young at the time of weaning were much greater. The weights of the young were less and the mortality greater than those obtained by supplementing natural foods with the vitamin G preparation. This indicates that another factor or factors aside from vitamins B and G is necessary for lactation. The preparations were fed at levels of 6 to 24 times the amount required for normal growth. The animals grew and reproduced normally but failed to rear their young. The unknown substance responsible for the favorable effects on lactation is not inorganic in character.

### 8134 P

## Blood Loss During Normal Menstruation.*

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*From the Department of Internal Medicine, State University of Iowa, Iowa City.*

During the course of iron balance studies on patients with hypochromic anemia, the amount of blood lost during menstruation was determined on several occasions. The figures obtained were surprisingly high but since no uniformity of opinion exists as to the normal menstrual loss an analysis of this was made on 50 women. The individuals selected were for the most part members of the hospital staff whose ages ranged from 19 to 43 years, who had no known menstrual disorders and whose blood hemoglobin was at least 75% of normal (10.2 gm. per 100 cc.).

Cellulo cotton pads were used in most cases although in a few instances a vaginal cup was utilized for the collection of the menstrual flow. This was analyzed for iron by a modification of the method of Reis and Chakmakjian,[1] and the result interpreted in terms of grams of hemoglobin on the assumption that this iron was derived entirely from hemoglobin.

Blood hemoglobin determinations were made on all subjects several days after the menstrual period to avoid variations that may occur because of changes in the water balance and the consequent hydremia. These values ranged from 10.200 to 13.190 gm. per 100 cc. of blood.

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* Supported in part by a grant from Eli Lilly & Co.

[1] Reis, F., and Chakmakjian, H. H., *J. Biol. Chem.*, 1931, **92**, 59.

The iron loss in these 50 individuals with supposedly normal menstrual periods varied from 3.84 to 78.4 mg. per period. This represents from 1.146 to 23.403 gm. of hemoglobin. If this is expressed in terms of cubic centimeters of blood, calculated in each instance from the patient's own hemoglobin content in grams per 100 cc., it represents a loss of 9.39 to 207.28 cc. with a mean of 36.7 cc. of blood per menstrual period. Much larger amounts of iron and hemoglobin were lost by the patients with hypochromic anemia even though they considered their menstrual periods to be entirely normal.

The loss of 78.4 mg. of iron at each menstrual period would necessitate a daily iron retention of approximately 2.8 mg. to replace that lost by menstruation alone. The results of iron balance studies, which are to be reported later, suggest that such a high iron retention is distinctly unusual with the average dietary intake of iron. This may explain the development of certain cases of idiopathic hypochromic anemia.

### 8135 C

## Effect of Low Carbohydrate Diet on Glucose Tolerance in Spontaneous Hypoglycemia.

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Since it was first pointed out by Gibson[1] that hypoglycemic symptoms not artificially produced may constitute a definite clinical entity, there has arisen considerable controversy as to the treatment of spontaneous hypoglycemia (or "hyperinsulinism"). Such views are that medical therapy is valueless and that relief of symptoms can be obtained only by surgical removal of a portion of the pancreas (or tumor of the pancreas[2]), frequent feedings of carbohydrates are indicated,[3] and that the diet should be low in carbohydrate and high in fat.[4] One investigator has suggested the use of insulin to diminish the glucose tolerance[5] which was observed to occur in insulinized non-diabetic patients by Paul, Clark, and Gibson.[6]

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[1] Gibson, R. B., and Larimer, R. N., *J. Am. Med. Assn.*, 1924, **82**, 468.

[2] Wilder, R. M., *International Clin.*, 1933, **2**, 1.

[3] Gammon, G. D., and Tenery, W. C., *Arch. Int. Med.*, 1931, **47**, 829.

[4] Harris, Seale, *J. Am. Med. Assn.*, 1933, **101**, 1958.

This communication reports the diminution of glucose tolerance and the relief of symptoms in 2 cases of mild to moderately severe spontaneous hypoglycemia with a low carbohydrate high fat diet.

TABLE I.
Effect of Low Carbohydrate Diet on the Glucose Tolerance in Spontaneous Hypoglycemia.

| Case 1. P.H. | | | Case 2. F.F. | | |
|---|---|---|---|---|---|
| Time | Blood Sugar | Remarks | Time | Blood Sugar | Remarks |
| 10-24-32 | | Mild symptoms | 10-4-32 | | |
| 8:00 | 66 | 50 gm. glucose | 5:00 | | 50 gm. glucose |
| 8:30 | 150 | | 8:00 | 75 | 50 " " |
| 9:00 | 159 | | 8:30 | 118 | |
| 9:30 | 124 | | 9:00 | 173 | |
| 10:00 | 98 | | 9:30 | 147 | |
| 10:30 | 55 | Hunger, weakness | 10:00 | 73 | |
| 10:45 | | 50 gm. glucose | 10:30 | 37 | Severe symptoms |
| 11:00 | 131 | Symptoms disap. | 11:00 | 55 | |
| 11:30 | 129 | | 11:30 | 59 | Symptoms milder |
| 12:00 | 118 | | 3-17-33 | Patient on high fat diet since 10-4-32 | |
| 12:30 | 52 | Severe symptoms | | | |
| 1:00 | 55 | | 8:00 | 78 | 50 gm. glucose |
| 1:30 | 71 | Symptoms milder | 8:30 | 189 | |
| 1:45 | | 10 gm. glucose | 9:00 | 226 | |
| 2:00 | 95 | Symptoms cleared | 9:30 | 217 | |
| Put on high fat diet 10-24-32 | | | 10:00 | 189 | |
| 10-11-33 | | | 10:30 | 170 | |
| 8:00 | 124 | 50 gm. glucose | 11:00 | 140 | 50 " " |
| 8:30 | 189 | | 11:30 | 124 | |
| 9:00 | 204 | | 12:00 | 204 | |
| 9:30 | 179 | | 12:30 | 200 | |
| 10:00 | 138 | | 1:00 | 170 | |
| 10:30 | 78 | Severe hunger | 1:30 | 142 | No symptoms during test |
| 10:45 | | 50 gm. glucose | | | |
| 11:00 | 114 | | | | |
| 11:30 | 170 | | | | |
| 12:00 | 155 | | | | |
| 12:30 | 104 | | | | |
| 1:00 | 79 | Slight hunger | | | |
| 1:30 | 77 | Tremor | | | |
| 2:00 | 95 | Feeling better | | | |
| High fat diet plus 3 slices bread since 10-11-33 | | | | | |
| 10-18-34 | | | | | |
| 8:00 | 112 | 50 gm. glucose | | | |
| 8:30 | 189 | | | | |
| 9:00 | 221 | | | | |
| 9:30 | 157 | | | | |
| 10:00 | 109 | | | | |
| 10:30 | 78 | | | | |
| 11:00 | 72 | Slight hunger and tremor | | | |
| 11:30 | 82 | Feeling better | | | |

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[5] John, H. J., *Endocrinology*, 1933, **17**, 583.

[6] Paul, W. D., Clark, B. B., and Gibson, R. B., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 353.

The clinical diagnosis and progress of these cases were confirmed by use of the double glucose tolerance test described by Hamman and Hirschman.[7] Blood sugars[8] were obtained every 30 minutes during the test. Two 50 gm. glucose meals were given 2¾ to 3 hours apart. The detailed account of these experiments is given in the following protocols, and the effect of the diet upon the glucose tolerance curves is summarized in Table I.

*Case 1.* A white male, aged 32, entered the University Hospital on 10-21-32. The symptoms were of 5 years' duration and consisted of attacks of heart palpitation which were preceded by nervousness, weakness, apprehension, shaking, perspiration and extreme hunger, occurring usually 2 to 3 hours after meals. The symptoms persisted in spite of treatment for heart disease and even after thyroidectomy. The physical examination was essentially negative. The basal metabolism was plus 14%.

The symptoms decreased after the patient had followed a daily diet of protein 60 gm., carbohydrate 60 gm., and fat 210 gm. for one year, and it will be noted from Table I that the glucose tolerance decreased. The maximum and minimum blood sugars on 10-21-32 were 159 and 52 mg. respectively, while on 10-11-33, one year later, they were 204 and 77 mg. The glucose tolerance and symptoms were practically unchanged after 3 slices of bread had been added to the diet. The patient reported on May 7, 1935, after 7 months on a general diet, that the frequency and severity of the previous attacks were again evident, particularly after a meal rich in carbohydrates.

*Case 2.* A white male, aged 48, entered the hospital on 9-29-32; had had attacks of weakness and jaundice at 3 to 4 month intervals for 6 years. The attacks of jaundice had gradually decreased while those of weakness had increased in frequency and severity. The weakness usually occurred 2 to 3 hours after meals and was accompanied by dizziness, but there was no history of perspiration, hunger or nervousness. The physical examination and laboratory findings revealed nothing of significance. The glucose tolerance, 5 days after admission, was increased with a minimum blood sugar of 37 mg. A daily diet of protein 60 gm., carbohydrate 60 gm., and fat 210 gm. was followed for 5 months with complete relief of symptoms and a marked diminution in glucose tolerance. The minimum blood sugar on 3-17-33 was the fasting one of 78 mg. and the maximum 226 mg. one hour after the first 50 gm. of glucose.

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[7] Hamman, L., and Hirschman, I. I., *Bull. Johns Hopkins Hosp.*, 1919, **30**, 306.

[8] Gibson, R. B., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 480.

The etiology of spontaneous hypoglycemia has been generally regarded as an over production of insulin, and while in a few cases tumors of the pancreas have been demonstrated,[9] other cases have shown normal pancreatic tissue.[10] Other possible causes of hypoglycemia should not be ignored. Experimental work has demonstrated hypophysectomized animals are very susceptible to spontaneous hypoglycemia[11] and to a less extent adrenalectomized animals.[12] The liver itself may be the controlling factor both physiologically[13] and pathologically.[14]

The medical treatment of hyperinsulinism with low carbohydrate high fat diets seems very rational. The decrease in glucose tolerance on such a diet was first shown by Sweeney[15] and verified by many workers; the experiments reported here show it clearly. That high carbohydrate or repeated ingestion of glucose under appropriate conditions stimulate the glucose tolerance has been shown in normals by Hamman and Hirschman[7] and others, and in diabetics by Gibson[16] as well as by others. It would seem logical then to expect that cases of hyperinsulinism would show a reduction of glucose tolerance and relief of symptoms by the use of a low carbohydrate diet, while certain other cases of spontaneous hypoglycemia may not respond to such treatment.

*Summary.* The marked improvement in symptoms and glucose tolerance curves in 2 cases of spontaneous hypoglycemia (hyperinsulinism) are reported following diets high in fat and low in carbohydrate.

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[9] Wilder, R. M., Allan, F. N., Power, M. H., and Robertson, H. E., *J. Am. Med. Assn.*, 1927, **89**, 348.

[10] Finney, J. M. T., and Finney, J. M. T., Jr., *Ann. Surg.*, 1928, **88**, 584.

[11] Houssay, B. A., and Biasotti, A., *Endocrinology*, 1931, **15**, 511.

[12] Barnes, B. O., Scott, V. B., Ferrill, H. W., and Rogoff, J. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 524.

[13] Soskin, S., Allweiss, M. D., and Cohn, D. J., *Am. J. Physiol.*, 1934, **109**, 155.

[14] Best, C. H., Ferguson, G. C., and Hershey, J. M., *J. Physiol.*, 1933, **79**, 94.

[15] Sweeney, J. S., *Arch. Int. Med.*, 1927, **40**, 818.

[16] Gibson, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 449; *J. Lab. and Clin. Med.*, 1929, **14**, 597.

### 8136 P

## Dextrose in Malnutrition.

GENEVIEVE STEARNS, RUTH CATHERWOOD AND ABRAHAM KANTROW.
(Introduced by P. C. Jeans.)

*From the Department of Pediatrics, State University of Iowa, Iowa City.*

It has been observed that children with malnutrition and an accompanying diarrhea usually have a markedly impaired ability to digest fats and complex carbohydrates, even though these children may not exhibit all the symptoms characteristic of celiac disease. In view of these findings it seemed possible that the ability to utilize fats and starches might be impaired in malnutrition of less severe degree.

To test this hypothesis, 5 children from 8-13 years old and from 10-20% underweight were used as subjects. These children were underweight presumably because of lack of proper food. Four of the 5 had hypertrophied tonsils; no other abnormality was observed. Three types of diet were used: a "normal diet," similar to the customary hospital diet; a "fat diet" containing increased amounts of butter and cream, and with a F.A:G ratio of 1.5:1, supposedly below the ketogenic level. The third or "dextrose diet" was the diet used in the treatment of celiac disease in this clinic; rich in protein, minerals and vitamins, very low in fat, and containing the carbohydrate almost wholly in the form of dextrose. The quantities of dextrose ingested daily varied from 220 gm. for the smallest child, to 445 gm. for the largest boy. Each child was given at least 2 diets, one child received each of the 3 in turn. The pertinent data as to gains in weight are shown in Table I.

TABLE I.
Gain in Weight in Relation to Type of Diet and Caloric Intake.

| | 40 to 50 cal. per lb. | | | | 50 to 60 cal. per lb. | | | |
|---|---|---|---|---|---|---|---|---|
| Type of Diet | No. periods | Av. days per period | Av. intake cal. per lb. per day | Av. gain lb. per day | No. periods | Av. days per period | Av. intake cal. per lb. per day | Av. gain lb. per day |
| "Fat" | 4 | 9 | 48.1 | .02 | 7 | 9 | 55.4 | .31 |
| Normal | 2 | 10 | 43.6 | .04 | 3 | 11 | 52.0 | .19 |
| Dextrose | 5 | 10 | 46.6 | .04 | 6 | 9 | 53.3 | .45 |
| | 4* | 12 | 46.6 | .17 | | | | |

*Omitting one 3-day period wherein diet was decreased 500 calories.

Consistent and satisfactory gains were rarely observed on any diet if the caloric intake was below 45 calories per pound body weight; regular gains were the rule whenever the daily intake was greater than 50 calories per pound. With any caloric intake, however, the largest and most consistent gains were made when the children were given the dextrose diet. To achieve a similar gain from the fat diet, from 250 to 500 additional calories per day were needed. Undoubtedly, during the first few days of the dextrose diet, a considerable portion of the weight gain could be ascribed to water retention; the gain thus made was not subsequently lost, however, and the very high nitrogen retentions observed indicate that this first water storage was soon replaced by nitrogenous tissue. When the children became normal in weight, a change to the general hospital diet did not occasion any loss of weight.

No child showed a glycosuria at any time during the study. One of the 5 children excreted more than 100 mg. of "acetone bodies" daily when given the "fat diet". Sugar tolerance curves at the end of each dietary period were as predicted; the highest rise of blood sugar after the fat diet, the lowest after the dextrose diet. No ill effects of any kind were observed from the ingestion of these large amounts of dextrose.

It is concluded that a diet rich in protein, minerals and vitamins, low in fat and containing its carbohydrate largely in the form of dextrose is best suited to produce rapid and consistent gain in weight of undernourished children. The caloric intake should be above 50 calories per pound of body weight.

## 8137 P

## Retention of Calcium by Infants Fed Evaporated Milk Containing Cod Liver Oil Concentrate.

P. C. JEANS AND GENEVIEVE STEARNS.

*From the Department of Pediatrics, State University of Iowa, Iowa City.*

This report concerns the quantities of calcium retained and the growth in length and weight of 5 white infants fed evaporated milk containing the unsaponifiable fraction of cod liver oil (Zucker concentrate) in an amount which allowed 400 U.S.P. units of vitamin D to the reconstituted quart. When the experiment was started one infant was 11 weeks, one 6 weeks, and the remaining 3 infants 10 to 20 days of age. The vitamin D intake of the youngest infants was 245 units daily; the maximum intake was 400 units a day. The intakes and retentions of calcium per kilogram of body weight are

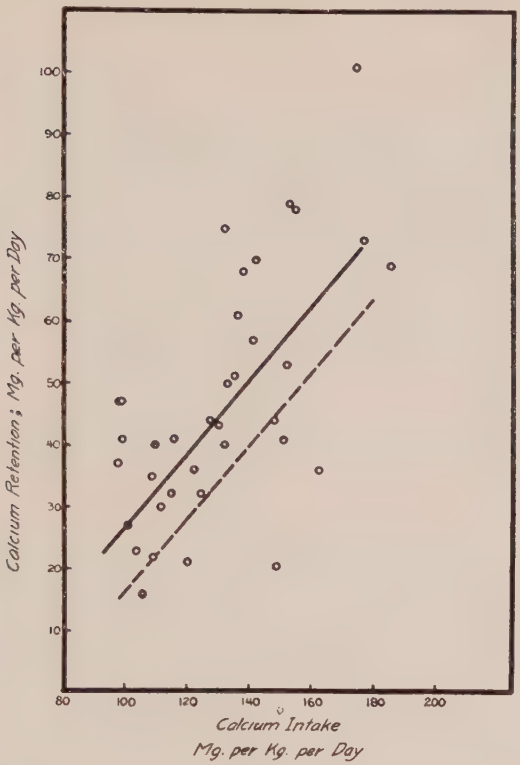

FIG. 1.

plotted on the chart. For comparison are shown average values from former observations. The solid line shows the average calcium retentions of infants given 340 U.S.P. units of vitamin D daily as cod liver oil and represents 200 periods of study of 24 infants; the dotted line shows the average retentions from 135 U.S.P. unit milk (60 to 135 units daily) as determined from 40 periods of study of 7 infants. The quantities of calcium retained from any given intake of the 400 unit milk were within the range observed in infants to whom 340 units of vitamin D as cod liver oil were given daily, and the average values for each intake range is approximately the same as that of the large control group, but about 10 mg. per kilo higher than the average retentions observed when 135 unit milk was given a similar group of infants.[1]

The serum calcium values varied from 10.4 to 12 and the serum phosphorus from 5.7 to 7.2 mg. per 100 cc.; the average values were 11.0 and 6.3 mg. per 100 cc., respectively. The rate of growth of each infant, both in length and weight, was above the Kornfeld[2] average, and equal to the average rate of growth of the infants given 340 units of vitamin D daily. No infant developed rickets; dentition was early and muscular development excellent.

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[1] Jeans, P. C., and Stearns, G., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1159.

It is concluded that milk containing cod liver oil concentrate (Zucker) sufficient to allow 400 U.S.P. units of vitamin D to the reconstituted quart, allows high retentions of calcium, prevents the development of rickets, and permits excellent growth and development of the infants.

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[2] Kornfeld, W., *Z. für Kinderheilkunde,* 1929, **48**, 202.

# Pacific Coast Section

*University of California, May 4, 1935.*

## 8138 C

## Bacterial Antifibrinolysins.*

MICHAEL DOUDOROFF. (Introduced by W. H. Manwaring.)

*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*

The specific antihuman fibrinolysin formed or secreted by certain strains of Streptococci is presumably one of the main factors causing the rapid spread of certain streptococcus infections on or within human tissues. The effect of associated bacterial species on this aggressive chemical factor, therefore, is of practical clinical interest.

To study such effects, an arbitrary dose of streptofibrinolysin was added to chloroformed broth cultures of the commoner associated microorganisms. At various times after this addition the fibrinolytic titers of the resulting mixtures were determined. Data thus obtained are recorded in Table I.

From the table it is seen that there is a suggestive parallelism between the destruction of the fibrinolysin and the power of the associated microorganisms to liquefy gelatin. Clinically important exceptions to this rule are: *Staph. aureus* (2 strains), *Cl. welchii* and *Cl. oedematiens-maligni,* which liquefy gelatin but are without demonstrable antifibrinolytic action.

The table further shows that the antifibrinolysin in certain bacterial cultures is partially or completely destroyed by heating to 60°C. for 30 minutes. All but one of the antifibrinolysins thus far tested are destroyed quantitatively by heating to 100°C. for 5 minutes.

Heating a non-lytic fibrinolysin-antifibrinolysin mixture to 100°C. for 5 minutes will not restore its fibrinolytic function. Such a

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* Work supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

### TABLE I.

Destruction of Strepto-fibrinolysin by Associated Bacteria.

Chamberland (L 3) filtrate of a 24-hour broth culture of a fibrinolytic strain of *Streptococcus hemolyticus,* heated to 100° C for 5 minutes to destroy possible living organisms, was diluted with 0.8% NaCl-solution to form a stock reagent containing 20 minimum fibrinolytic units per cc., the lytic unit being determined by the serum-free fibrin-clot technic of Tillett and Garner.[1] Samples of this stock lysin were added to equal volumes of 48-hour broth cultures of the microorganisms to be tested and a drop of chloroform added to each mixture to inhibit bacterial growth. The mixtures were incubated at 37° C for 24 hours, and the rate of destruction of the fibrinolysin determined by the serum-free fibrin-clot technic.

The following arbitrary symbols are used to record results: ++++, cultures causing at least 90% destruction of the fibrinolysin by the end of one hour; +++, by the end of three hours; ++, six hours; +, 24 hours; ±, 50% destruction by the end of 24 hours; and 0 no demonstrable reduction in fibrinolytic titer.

| Bacterial strains tested | Antifibrinolytic titer | Titer of heat-inactivated cultures 60°, 30 min. | Titer of heat-inactivated cultures 100°, 5 min. | Gelatin liquefaction |
|---|---|---|---|---|
| Non-fibrinolytic *Strep. hemolyticus* (8 strains) | 0 | 0 | 0 | 0 |
| Non-fibrinolytic *Strep. hemolyticus* (1 strain) | ++++ | ± | 0 | + |
| Non-hemolytic Streptococci (26 strains) | 0 | 0 | 0 | 0 |
| *Strep. viridans* (2 strains) | ± | 0 | 0 | 0 |
| *Strep. liquefaciens* (1 strain) | +++ | 0 | 0 | + |
| *Strep. liquefaciens* (1 strain) | ++++ | + | 0 | + |
| *Staphylococcus albus* (1 strain) | 0 | 0 | 0 | 0 |
| *Staphylococcus aureus* (1 strain) | 0 | 0 | 0 | 0 |
| *Staphylococcus aureus* (2 strains) | 0 | 0 | 0 | + |
| *Dip. pneumoniae* (5 strains) | 0 | 0 | 0 | 0 |
| *Kleb. pneumoniae* (2 strains) | 0 | 0 | 0 | 0 |
| *C. diphtheriae* (2 strains) | 0 | 0 | 0 | 0 |
| *Esch. coli* (2 strains) | 0 | 0 | 0 | 0 |
| *Eberth. typhi* (1 strain) | 0 | 0 | 0 | 0 |
| *S. dysenteriae* (1 strain) | 0 | 0 | 0 | 0 |
| *Proteus vulgaris* (2 strains) | 0 | 0 | 0 | ± |
| *Proteus vulgaris* (1 strain) | +++ | ++ | + | + |
| *Ps. fluorescens* (1 strain) | ++++ | ++++ | 0 | + |
| *Ps. aeruginosa* (2 strains) | ++++ | ++++ | 0 | + |
| *Ps. cyanogena* (1 strain) | 0 | 0 | 0 | 0 |
| *B. anthracis* (1 strain) | ++ | 0 | 0 | ± |
| *Cl. welchii* (1 strain) | 0 | 0 | 0 | + |
| *Cl. oedematiens-maligni* (1 strain) | 0 | 0 | 0 | + |
| *Cl. sporogenes* (1 strain) | ++++ | ++++ | 0 | + |
| *Cl. histolyticum* (1 strain) | ++++ | ++ | 0 | ± |

restoration would be logical if the coctostable fibrinolysin were merely bound or inhibited by the coctolabile antifibrinolysin.

None of the associated bacteria thus far tested increase the lytic action of streptofibrinolysin.

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[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

### 8139 C

## Specificity of Hybrid Proteins.*

AUGUST G. ZOET. (Introduced by W. H. Manwaring.)

*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*

As a result of crossing female domestic Ring doves with males of an Asiatic species, Irwin[1] reports the emergent evolution of a new protein specificity in hybrid doves, no trace of which is demonstrable in the erythrocytes of either parent.

To throw light on the probable immunochemical mechanism involved in this apparent non-conformity with Mendelian laws, we have tried to produce analogous test-tube hybridizations between proteins of 2 widely different animal species.

Serum proteins of the horse and domestic swine were selected for this purpose. To bring about rapid hybridization between these proteins, horse serum and swine serum were mixed in equal proportions, the resulting mixture diluted with 3 volumes of distilled water and then heated to 120°C. for 30 minutes. After cooling, the

TABLE I.

Titration with Antihybrid Rabbit Precipitin.

A. First Column: 0.2 cc. 50% antihybrid rabbit serum, plus 0.2 cc. serial dilutions of horse-swine hybrid proteins; incubator 2 hours; ice-chest over night; resulting precipitates estimated quantitatively (+, ++, +++, ++++). t, opalescence without demonstrable precipitate. 0, no demonstrable reaction.

Second column: Parallel tests with a fresh (*i. e.,* non-hybridized) mixture of the control preparations of horse serum and swine serum.

B. Retests with the same antiserum after specific absorption or removal of the anti-horse and anti-swine precipitins by incubating for 2 hours in the presence of a 1:80 dilution of a fresh (*i. e.,* non-hybridized) mixture of horse serum and swine serum.

| Serial dilution of antigen | A. Tests with native antihybrid precipitin | | B. Parallel tests with reduced antihybrid precipitin | |
|---|---|---|---|---|
| | Horse-swine hybrid proteins | Horse serum + swine serum | Horse-swine hybrid proteins | Horse serum + swine serum |
| 1:40† | ++++ | + | ++++ | t |
| 1:80 | ++++ | + | ++++ | 0 |
| 1:160 | ++++ | 0 | ++++ | 0 |
| 1:320 | +++ | 0 | + | 0 |
| 1:640 | t | 0 | t | 0 |
| 1:1280 | t | 0 | 0 | 0 |
| 1:2560 | 0 | 0 | 0 | 0 |

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*Work supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Irwin, R. R., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 850.

†To change these dilutions into conventional titers, multiply the denominators by 10.

osmotic pressure was restored to normal by the addition of NaCl. As controls, unmixed horse serum and swine serum were similarly diluted, heated and restored to normal osmotic pressure.

Rabbits were immunized against the artificial horse-swine hybrid serum. A typical set of serological tests with the resulting anti-hybrid precipitin is recorded in Table I.

From the table it is seen that the artificial horse-swine hybrid serum contains one or more specific factors not present in horse serum or swine serum.

If an analogous fusion of paternal and maternal protein specificities takes place during sexual reproduction, the apparent emergent evolution of one or more new protein characters in certain hybrids would be in accord with Mendelian laws.

The above data are consistent with the current theory that the specific antigenicity of a given biological product is not determined solely by its specific haptene content, but is largely dependent on the topographical distribution of these haptenes on or within colloidal molecules.

### 8140 C

## Iodine Values and Total Lipids of Leprous Human Blood Sera.*

HAMILTON H. ANDERSON AND JEANETTE VAN D. ANDERSON.

*From the Pacific Institute of Tropical Medicine, Hooper Foundation for Medical Research, and the Pharmacologic Laboratory, University of California Medical School, San Francisco.*

Recent renewed interest in serum lipid changes relative to the therapeutic effects of various unsaturated oils in skin diseases[1] prompted us to determine the iodine values and serum lipids of leprous patients receiving chaulmoogra therapy. Unsaturated oils, in addition to those of the chaulmoogra group, have been used with some success in leprosy, notably olive, cod liver, dilo, soy bean, margosa, and coconut, according to Cole.[2] De Vera has shown that in a series of oils of diminishing saturation, the greater the unsaturation, the greater is the therapeutic action.[3] Walker and Sweeny[4] demonstrated that unsaturation is but partly responsible for the *in vitro* activity of non-chaulmoogra oils. The mechanism of action of non-chaulmoogra unsaturated oils has been studied in other skin diseases recently. Hansen found the iodine values of the blood in infantile eczema to be abnormally low. By administering linseed oil in large amounts an increase of the degree of unsaturation was noted which paralleled clinical improvement.

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* In cooperation with the Health Department of the Panama Canal and the Gorgas Memorial Laboratory, Panama; through the courtesy of Dr. H. C. Clark, Col. J. F. Siler, Dr. D. P. Curry, Dr. Ezra Hurwitz, and Mr. Everett Jacob. Supported in part by Eli Lilly and Co., Indianapolis, Indiana.

[1] Hansen, A. E., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 1198; *ibid.*, 1933, **31**, 160, 161.

[2] Cole, H. I., *Intern. J. Leprosy*, 1933, **1**, 159.

The blood plasma lipids in leprosy have been studied by Paras[5] working at Culion in the Philippine Islands. Quoting Rogers, who suggested an increased lipolytic activity of the blood as a result of treatment, Paras postulates that the blood lipoids would tend to increase in rapidly improving cases of leprosy, in which the mycobacteria are presumably destroyed in large numbers. In untreated and treated groups showing little or no improvement, and in which the mycobacteria continue to multiply, the blood lipoids would tend to remain low. While Paras' findings did not support his hypothesis he felt that the possibility was of sufficient importance to warrant further study. He found, incidentally, in untreated cases low values for total blood lipoids, and in treated ("positive") cases, values but slightly lower than normal were noted, while in treated ("negative") patients the lipoids were within normal limits. One of the possibilities suggested by these findings was, according to Paras, "that the leprosy bacilli attempt to protect themselves from the destructive action of the defense mechanism of the body by utilizing part of the plasma lipoids".

We have had an opportunity to examine the blood sera of patients with "cutaneural" (*i. e.,* mixed) leprosy at the Palo Seco Leper Colony at Panama. Determinations of fatty acid unsaturation in terms of iodine values were made following the method of Page, Pasternak, and Burt[6] and are shown in Table I together with the total lipids in various groups of patients. Since anemia, malnutrition, and nephritis are thought to cause lipemia,[7] average hemoglobin findings and weights are recorded. All patients were examined for evidence of kidney damage and those reported had

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[3] de Vera, B., *J. Phil. Isl. Med. Assn.*, 1925, **5**, 374.

[4] Walker, E. L., and Sweeney, M. A., *J. Infect. Dis.*, 1920, **26**, 238.

[5] Paras, E. M., *J. Phil. Isl. Med. Assn.*, 1931, **11**, 1.

[6] Page, I. H., Pasternak, L., and Burt, M. L., *Biochem. Z.*, 1930, **223**, 445.

[7] Peters, J. P., and Van Slyke, D. D., Qualitative Clinical Chemistry, Vol. 1, Interpretations, Williams and Wilkins Co., Baltimore, 1931.

TABLE I.

Average Iodine Values and Total Lipids of Blood Sera of 53 Patients under Treatment for "Cuta-neural" (Mixed) Leprosy at Palo Seco Leper Colony, Panama.[†]

| Stage and course of disease | No. of patients | Av. age in years | Av. wt. in kilos | Av. Hgb. Gm./100 cc. | Probable av. duration of disease in years | Probable av. amount of Chaulmoogra esters given in kilos | Iodine value | Total lipids Gm./100 cc. |
|---|---|---|---|---|---|---|---|---|
| Early predominantly cutaneous leprosy[‡] | 18 | 30.1 | 57.2 | 12.15 | 4.1 | 1.44 | 50.1 ±13.6 | 0.995 ±0.129 |
| All stages—improving[§] | 13 | 30.4 | 59.9 | 11.85 | 5.3 | 1.57 | 54.5 ±18.1 | 0.813 ±0.194 |
| Advanced—stationary | 11 | 31.9 | 56.2 | 11.02 | 9.4 | 2.25 | 66.9 ±14.1 | 0.720 ±0.200 |
| All stages—advancing | 11 | 33.1 | 58.7 | 12.67 | 9.8 | 2.74 | 77.8 ±19.8 | 0.572 ±.0148 |

[†] Normal values: Total lipids, 0.589 Gm./100 cc.; Iodine value of total fatty acids, 88. (Boyd, E. M., *J. Biol. Chem.*, 1933, **101**, 323.)
Average values for 53 leprous patients: Total lipids, 0.805 ±0.161 Gm./100 cc. Iodine value, 58.5 ±15.9.

[‡] Three patients untreated.

[§] One patient with lepra fever. Iodine value, 31.2; total lipids, 1.016 Gm./100 cc.

normal phenolsulphonphthalein excretion. The level of total lipids supposedly increases with age, but our data do not show this trend in leprosy. All patients exhibited acid-fast organisms in the nasal mucosa or in skin biopsy material.

Analyzing the findings presented, we note abnormally low iodine values in early "cuta-neural" leprosy, 3 untreated cases exhibiting numbers of 40.0, 41.6, and 44.4 respectively. The total lipids are above the normal range. Clinically improving cases have slightly higher iodine values and lower total lipids, which might be explained by the higher average dosage of chaulmoogra esters given. Advanced treated cases of leprosy show greater unsaturation of the blood fatty-acids and lower total lipids, but again the findings are masked by the large amounts of chaulmoogra administered.

A possible explanation of the decrease in unsaturated blood fatty acids in the early leprosy is that since the *Mycobacterium leprae* requires such substances in its metabolism there may occur a gradual depletion in the tissues of these unsaturated acids, and a resultant lowering of the blood levels as equilibrium is reached. There does not seem to be a marked increase in iodine values in cases improving under extensive treatment with chaulmoogra esters as has been noted in infantile eczema treated with linseed oil. In fact, the 3 highest numbers—108.0, 120.4, and 129.3—occurred in treated patients rapidly becoming worse. This might indicate that the unsaturated chaulmoogric acids (iodine value of U.S.P. chaulmoogra oil 98-104) are utilized by the *Mycobacterium leprae* or are converted into a substance suitable for its growth and development. Such a circumstance would prevent the concentration of unsaturated fatty acids in the blood of patients receiving relatively large amounts of chaulmoogra.

*Summary.* The blood of 53 "cuta-neural" (*i. e.,* mixed) cases of leprosy were examined. The average iodine value for the group was 58.5 ± 15.9, the total lipids being 0.805 ± 0.161 Gm./100 cc. Early "cuta-neural" leprosy and cases improving under chaulmoogra therapy exhibited higher unsaturation of the fatty acids but lower lipids than did the blood of more extensively treated advanced cases which were stationary or progressing.

## 8141 C

## Testicular Hormones and Sebright Plumage.*

C. H. DANFORTH.

*From the Department of Anatomy, Stanford University.*

There are recognizable in the fowl 3 intergrading types of plumage, usually designated as hen-feathering, cock-feathering, and capon-feathering. The capon type, which does not occur normally in nature is, in effect, an exaggeration of the male form. It appears following the complete removal of testes or ovaries. Hen-feathering occurs in the presence of an adequate amount of functional ovarian tissue (native or grafted) in any individual, so far as known, without regard to breed or sex. It may also be induced by the injection of certain hormones, such as thyroxin, and occurs normally in males of certain breeds, notably Sebrights.

Attempts to interpret these phenomena have led to 2 not wholly concordant views. One group of investigators[1] has postulated differences in testicular hormones, pointing out that the degree of cock-feathering varies in different races, but that in all races the male is less elaborately feathered than the corresponding capon. Others have questioned the differential effect of testicular hormones on plumage. Roxas[2] found that exchange of testes between hen-feathered and cock-feathered males had no effect on the plumage of either. Gallagher, Domm and Koch[3] have recently reported that purified testicular hormone, while having no effect on capons of cock-feathered strains, readily induces hen-feathering in Sebright capons. They interpret these findings as indicating merely somatic differences in response to the same hormone. Results following skin transplantation,[4] while for the most part supporting this contention, afford some indication of racial differences in the hormones themselves. The question is obviously still open, and calls for a more nearly quantitative analysis. The following experiments only partially meet this need.

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*Supported in part by a grant from the Rockefeller Fluid Research Fund to the Stanford University School of Medicine.

[1] Benoit, J., *Arch. Zool. Exper.*, 1932, **73**, 1; Pezard, Sand et Caridroit, *Arch. de Biol.*, 1926, **36**, 541; Morgan, T. H., *Carnegie Inst. Washington Pub.*, **258**.

[2] Roxas, H., *J. Exp. Zool.*, 1926, **46**, 63.

[3] Gallagher, T. F., Domm, L. V., and Koch, Fred C., *J. Biol. Chem.*, 1933, **100**, xlvii.

[4] Danforth, C. H., *Biol. Gen.*, 1930, **6**, 99.

Three 5½-months-old cockerels of Sebright type, full brothers, and very similar in appearance, were used for a gonadectomy test. In *A* the testes were left undisturbed, in *B* both were removed, and in *C* one was removed. *A* and *C* remained hen-feathered and normal as to comb and reactions; *B* became a typical capon with abundant plumage. After a year the greater part of the remaining testis was removed from *C*, following which his comb diminished and he became capon-like in behavior and plumage. A few months later, however, his comb returned to normal, he became masculine in behavior and acquired a fairly good type of cock-feathering. *C* remained in appearance a cock-feathered male till he was killed some 3 years later. All 3 birds were autopsied at about 5 years of age. *A* had normal testes, *B* no testicular tissue, and *C* a small nodule. In plumage they were, respectively, hen-feathered, capon-feathered and cock-feathered. *A* and *C* had normal combs and had been cocky in bearing, *B* was a typical capon. This would seem to indicate that the type of plumage produced by a Sebright soma may be directly associated with the amount of functional Sebright testicular tissue; that in this case there is a purely quantitative relation between testicular output and type of plumage.

Another capon of Sebright type was grafted on the breast with bits of testis from a cock-feathered strain. After a few months his comb increased appreciably and he acquired a plumage that was definitely more that of a cock than that of a capon. With minor fluctuations, he continued in this condition for two years, at the end of which time he was given injections of a potent extract of beef testis on two successive days. Feathers developing at the time of these injections showed characteristic diamond shaped "feminized" areas, which is quite in agreement with the findings of Gallagher, Domm and Koch. This bird had a graft of female skin (bearing at the time feathers of male type) which responded to the testicular hormone even more definitely than did the skin of the host. In this case it would seem that derivatives from testes of bulls and of cock-feathered male fowls may be made to augment each other in the production of hen-feathering in a Sebright. Finally, after the effects of this treatment had worn off, the same bird was given daily injections of 200 rat units of gonadotropic hormone[5] over a period of 17 days, at the end of which time testicular material weighing about a gram and showing all stages of spermatogenesis was removed from the breast. Feathers developed during the period of gonadotropic injections were influenced in the same direction, and to about the same degree as those influenced by beef testes. They fell short of being fully of the hen type. Following removal of the grafted testis new feathers were of the capon type, similar to those developed before testicular grafting or injection. Following a second testicular graft, feathers completely of the hen type were produced by both grafted and host skin.

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[5] I am greatly indebted to Dr. H. H. Cole for a very generous supply of gonadotropic hormone.

These observations are essentially in accord with the findings (but not necessarily with the conclusions) of most students of this subject. It appears to the present writer that there are probably both somatic and endocrine variables, that the testicular secretion of hen-feathered and cock-feathered male are not precisely equivalent, at least quantitatively, and that thresholds of response are not so distinct as has sometimes been supposed. Further, the rather similar manifestations following injection of mammalian testicular extract and excessive stimulation of an avian testicular graft does not afford evidence of sufficient delicacy to warrant the conclusion that the bull and cock produce identical testicular hormones.

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## 8142 C

## Two Gonadotropic Substances in Mare Serum.*

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*From the College of Agriculture, University of California, Davis.*

The finding by Cole and Hart[1] that there is no relationship between the activity of mare serum when administered alone and its activity when combined with pituitary synergist,† led them to believe that there are two gonadotropic substances in mare serum. More convincing evidence is now at hand. We have been able to make potent pregnancy serum completely ineffective when given alone without destroying its synergistic properties. This has been accomplished by several diverse methods. After treatment with $H_2S$ and subsequent incubation at 37°C., the serum when given alone has no effect on the ovaries of immature rats, but when this serum is combined with pituitary synergist, marked augmentation occurs as is shown in Table I.

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* The chemical work described here was carried out in the Department of Biochemistry of the University of California under the supervision of Dr. D. M. Greenberg.

[1] Cole, H. H., and Hart, G. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 370.

† This pituitary synergist was prepared after the method of Evans *et al.*[2] and probably is an impure preparation of the follicle-stimulating hormone of Fevold and Hisaw.

[2] Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 545.

TABLE I.

| Material | | Amount injected | Ovarian weight, mg. |
|---|---|---|---|
| Serum treated with $H_2S$ not incubated | | 1 cc. | 163 |
| ” ” ” $H_2S$ and ” | | 1½ cc. | 13 |
| Pituitary synergist | | 2 mg. | 25 |
| Serum treated with $H_2S$ and incubated | combined | 1½ cc. | |
| Pituitary synergist | | 2 mg. | 48 |

We interpret this as meaning that one substance has been destroyed by this treatment. The substance destroyed was capable of affecting the ovary either alone or in conjunction with the luteinizer remaining in the serum after treatment.

We have attempted to separate the 2 hormones from mare serum without destruction of either as has been done so successfully with the pituitary by Fevold and Hisaw.[3] They have shown that the pituitary contains 2 gonadotropic substances, a follicle-stimulator and a luteinizer. According to them the follicle-stimulating hormone will give a moderate response consisting of follicular development when given alone and the luteinizer gives no increase in ovarian weight, whereas if the 2 are combined the response is augmented. By Fevold's method, as well as by the method just described, we have succeeded in obtaining fractions of mare serum which behave similarly to pituitary luteinizer; *i. e.,* the fraction when administered alone gives no response but if united with pituitary synergist, the response of the latter is augmented. This is shown in Table II.

TABLE II.

| Material | | Amount injected mg. | Ovarian weight mg. |
|---|---|---|---|
| Mare serum luteinizer | | 10 | 18 |
| Pituitary synergist | | 2 | 25 |
| Mare serum luteinizer | combined | 5 | 63 |
| Pituitary synergist | | 2 | |

We were also able to effect a crude concentration of the luteinizing substance by salting out, a method suggested by the work of

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[3] Fevold, H. L., and Hisaw, F. L., *Am. J. Physiol.,* 1934, **109,** 655.

Goss and Cole.[4] We separated out 4 fractions of serum. All of these stimulated the ovaries of immature rats when given by themselves but only one of these fractions, that salted out by the addition of between 14% and 18% $Na_2SO_4$, was capable of augmenting the effect of pituitary synergist, showing that most of the luteinizing substance was contained in this fraction.

Thus, our data show that pregnant mare serum contains a luteinizer‡ identical, or at least very similar, to that occurring in the pituitary together with a second gonadotropic hormone which is destroyed after treatment with $H_2S$ and subsequent incubation. We have not been able to identify this second substance as the follicle-stimulating hormone of Fevold and Hisaw. None of our fractions have given a pure follicle-stimulating response and we have obtained only slight evidence of augmentation when serum luteinizer has been combined with other mare serum fractions. In fact, we have not been able to differentiate the response of serum fractions which are effective when administered alone from the response of untreated serum. Our failure to obtain a fraction of mare serum having biological properties comparable to those of the follicle-stimulating hormone of Fevold and Hisaw may be explained by assuming either that the second gonadotropic substance in mare serum is not identical to the follicle-stimulating hormone obtained from the pituitary or that a follicle-stimulating substance similar to that found in the pituitary is present but not recognized because of contamination with serum luteinizer. Our data would indicate that the first assumption is the correct one.

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[4] Goss, H., and Cole, H. H., *Endocrinology*, 1931, **15**, 214.

‡ This so-called serum luteinizer is probably also identical to the substance which Evans *et al.* found in the sera of non-pregnant animals and referred to as a "prolan-like" substance.

### 8143 P

# Action of Dinitrophenol on Rate of Oxidation of Ethyl Alcohol in Vitro.*

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It has recently been shown by Widmark[1] that the injection of dinitrophenol in a dose of 10 mg. per kilo in dogs is effective in increasing the rate of disappearance of a previously administered dose of alcohol from the blood stream. He was not certain whether this was due to an increase in the rate of alcohol-metabolism from the drug itself, or to an increased rate of metabolism due to the hyperthermia, or to an increased elimination by the lungs from hyperventilation. Similar results have been reported by Harger and Hulpieu.[2] That alcohol may be oxidized in the presence of tissues *in vitro* has long been known.[3] We therefore determined whether dinitrophenol could increase this rate of alcohol oxidation *in vitro* by tissues, since this fact was indispensable in deciding as to the mechanism of the increased rate of fall of the blood alcohol concentration. Preliminary control experiments showed that dinitrophenol did not oxidize alcohol directly in the absence of tissues in concentrations used in the present study.

The technic of Le Breton[4] was used, modified to adapt it to the alcohol method of Cannan and Sulzer.[5] Male white rats of 250 to 300 gm. were killed by a blow on the head and decapitated. The liver was removed, and sections 0.5 mm. in thickness prepared immediately; 300 mg. of the liver slices was placed in a 25 cc. Ehrlenmeyer flask, 3 cc. of 0.1% ethyl alcohol in phosphate buffer of pH 7.4, and 0.3 cc. of the appropriate dilution of dinitrophenol added, and the air displaced by oxygen. Each determination was run in duplicate, with duplicate controls from the same animal, which differed only in the absence of dinitrophenol. The 4 flasks were incubated at 37°C. for 2 hours, being shaken mechanically 120

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* Supported, in part, by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Widmark, E. M. P., *Biochem. Z.*, 1935, **276**, 268.

[2] Harger, R. N., and Hulpieu, H. R., *Science*, 1935, **81**, 6.

[3] Battelli, F., and Stern, L., *Compt. Rend. Soc. Biol.*, 1909, **67**, 419; Hirsch, J., *Biochem. Z.*, 1916, **77**, 129.

[4] Le Breton, E., *Compt. Rend. Soc. Biol.*, 1935, **118**, 64.

[5] Cannan, R. K., and Sulzer, R., *Heart*, 1924, **11**, 148.

times per minute during this period. They were then placed in ice water to stop the oxidation, and the alcohol content determined.

Table I summarizes the results obtained, the rate of alcohol-metabolism being expressed in gm. per kilo of liver per hour. The last column gives the results for change in oxygen consumption as determined by McCord,[6] for comparison. Each value is the average of the duplicate determinations.

TABLE I.

| End Concentration Dinitrophenol | Rate of Alcohol Combustion |  | % Change | Change in Oxygen Consumption (McCord) % |
|---|---|---|---|---|
|  | D.N.P. | Control |  |  |
| 1:100,000 |  |  |  | —20 |
| 1:200,000 | 1.198 | 1.222 | —2 |  |
|  | 1.629 | 1.748 | —7 |  |
|  | 1.749 | 1.844 | —5 |  |
| 1:1,000,000 | 1.797 | 1.869 | —4 | —5.7 |
| 1:5,000,000 | 1.318 | 1.222 | +8 | —7.9 |
| 1:20,000,000 | .814 | .767 | +6 | +18.4 |
|  | 1.461 | 1.318 | +11 |  |
|  | 1.461 | 1.437 | +2 |  |
|  | 1.293 | 1.221 | +6 |  |

It will be seen that the changes, though small, were consistent. The dinitrophenol increased the oxidation of the alcohol in concentrations of 1:5,000,000 and 1:20,000,000. This stimulating concentration range was of the same order of magnitude as that found by McCord for stimulation of the oxygen consumption, in a medium of the same pH.

*Conclusion.* At a pH of 7.4, concentrations of dinitrophenol from 1:5,000,000 to 1:20,000,000 slightly increased the rate of oxidation of alcohol by rat liver *in vitro,* while higher concentrations slightly diminished it. This indicated that under some conditions an increase in tissue metabolism produced by dinitrophenol is accompanied by an increased rate of oxidation of alcohol. From this it may be deduced that the increased rate of fall of blood alcohol concentration caused by dinitrophenol in animals may be accounted for, in part at least, by an increased rate of its oxidation in the tissues. Further work, both *in vitro* and *in vivo,* is in progress to investigate this problem more fully.

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[6] McCord, W. M., *Am. J. Physiol.,* 1934, **109**, 232.

8144 C

# Comparison of Resistance of Bacteria and Embryonic Tissue to Germicidal Substances. V. Iodine.

A. J. SALLE AND A. S. LAZARUS.

*From the Department of Bacteriology, University of California, Berkeley, California.*

Lambert[1, 2] immersed fragments of human tissues in a dilute broth culture of *Staphylococcus aureus* for a few minutes and then in various dilutions of iodine for one hour. The tissue fragments were washed in physiological salt solution, then embedded in plasma and examined for growth of bacteria and tissue after several days of incubation. He found that human connective tissue cells and histiocytes were, in general, more easily killed than *Staphylococcus aureus*. Of the compounds tested iodine approached most closely the "ideal" disinfectant, killing bacteria in strengths that did not seriously injure human connective tissue cells or wandering cells.

Lambert and Meyer[3] placed fragments of rabbit spleen in a suspension of *Staphylococcus aureus* for one minute followed by immersion in graded solutions of iodine for 20 minutes. Tissue cultures were then prepared in hanging drops of homologous plasma, following 2 washings of the tissue in physiological salt solution. A second set of hanging drop cultures was made from non-infected tissues similarly exposed with appropriate controls of untreated tissues. They found that some of the classic germicides such as iodine and mercuric chloride approached nearer the "ideal" than certain of the newer preparations.

Buchsbaum and Bloom[4] prepared chick tissue cultures in which the various dilutions of iodine were embedded in chick plasma. The test organism, *Staphylococcus aureus,* was added to the embryonic fluid. They stated that an antiseptic killing the bacteria at concentrations that would not harm the cells would have an index of 1.0 or greater. Iodine was given an index of 0.5.

In previous papers of this series,[5-8] comparisons were made of the

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[1] Lambert, R. A., *J. Exp. Med.*, 1916, **24**, 683.

[2] Lambert, R. A., *J. A. M. A.*, 1916, **67**, 1300.

[3] Lambert, R. A., and Meyer, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 429.

[4] Buchsbaum, R., and Bloom, W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1060.

[5] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 665.

resistance of *Staphylococcus aureus* and embryonic chick heart tissue to phenol, Merthiolate, Metaphen, Mercurochrome and Hexylresorcinol. A *Staphylococcus aureus* phenol coefficient and a toxicity index were determined for each germicide. The methods followed were the same as those described in the first communication.[5]

The highest dilution of phenol required to kill *Staphylococcus aureus* in 10 minutes but not in 5 minutes was 1:65. For iodine* it was 1:20,000. This gave iodine a *Staphylococcus aureus* phenol coefficient of 308.

Birkhaug,[9] employing Tincture of Iodine U. S. P., reported a *Staphylococcus aureus* phenol coefficient of 38 by the same method.

The tissue culture results are summarized in Table I.

TABLE I.

| Germicide | Highest dilution showing no tissue growth = A | Highest dilution showing no growth of *Staph. aureus* = B | Toxicity index = A/B | *Staphylococcus aureus* phenol coefficient |
|---|---|---|---|---|
| Phenol | 1:840 | 1:65 | 12.9 | |
| Iodine | 1:1,800 | 1:20,000 | 0.09 | 308 |

The results of tests obtained from all of the germicides so far completed are given in Table II.

TABLE II.

| Germicide | Toxicity index | *Staphylococcus aureus* phenol coefficient |
|---|---|---|
| Iodine | 0.09 | 308 |
| Hexylresorcinol | 3.0 | 108 |
| Metaphen | 12.7 | 92 |
| Phenol | 12.9 | — |
| Merthiolate | 35.3 | 70 |
| Mercurochrome | 262.0 | 0.6 |

Theoretically the smaller the toxicity index the more nearly perfect the chemotherapeutic agent. It is seen from the above results that iodine is far superior to all of the chemicals so far studied when tested by the tissue culture technique. It gave not only a smaller toxicity index but a greater *Staphylococcus aureus* phenol coefficient.

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[6] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 937.

[7] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1057.

[8] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1119.

* Lugol's Solution (Liquor Iodi Compositus, U. S. P.) was used in all tests.

[9] Birkhaug, K. E., *J. A. M. A.*, 1930, **95**, 917.

Lambert[1] stated: "An ideal antiseptic is obviously one that will kill the infecting agent without at the same time injuring body cells."

"Iodine stands out as the one chemical tested to which cells were found to be more resistant than were Staphylococci. A good growth of cells was seen after exposure to a 1:2,000 solution of iodine for one hour, a strength sufficient to sterilize the tissue completely in most instances."

"These experiments afford further experimental evidence of the value of iodine as an antiseptic, and indicate that, at least in weak aqueous solution it should not, as is often stated, injure or irritate the tissues."

Our results are in harmony with those obtained by the above worker.

# Southern Section

*Tulane University, May 17, 1935.*

## 8145 P

## Experimental Propagation of Strongyloides in Culture.*

TED DE VINNE BEACH. (Introduced by Ernest Carroll Faust.)

*From the Parasitology Laboratory, Department of Tropical Medicine, Tulane University, New Orleans, La.*

The nematode parasite *Strongyloides simiae* of New World monkeys has as a part of its life cycle a free-living phase outside the body of the host. The eggs laid by the parasitic females are passed in the feces, rhabditiform larvae hatched from them feed on bacteria and debris, and under suitable conditions, metamorphose into free-living, sexually mature forms. These adults mate and produce eggs and larvae supposedly identical with those of the first generation. Such second generation individuals have been considered capable only of developing into filariform larvae. Leuckart's[1] views on the fate of the second generation larvae probably have influenced the general belief that only one sexual generation of the free-living phase could develop. The only statement regarding the probable continued propagation of the free-living phase of this organism was that by Faust,[2] who predicted indefinite free-living development.

By planting a known number of *S. simiae* eggs from the stool of *Cebus capucinus imitator* which harbored the parasitic females, on a culture medium consisting of a nutrient agar, 2 gm., filtered, aqueous extract of monkey feces, 25 cc., and distilled water, 75 cc., it was possible to observe the entire developmental cycle of larvae and adults and their descendants.

In 4 experiments using the above mentioned medium, free-living sexually mature second generation males were produced on 11 cul-

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* Supported by a grant from the Committee on Scientific Research, American Medical Association.

[1] Leuckart, R., 1882.

[2] Faust, E. C., Human Helminthology, Phila., 1929, p. 34.

tures. Five cultures produced second generation females as well. Both sexes of the second generation instead of metamorphosing into filariform larvae retained their rhabditiform characteristics. The males widened posteriorly and spicules appeared. At first no eggs were seen in the young females but with an enlargement of the vulva, eggs appeared in the uterus. In some instances the first generation adults were dead before those of the second generation matured. In most cases, however, the former were present with the latter but soon died while those of the second generation persisted and laid eggs which hatched and developed into third generation filariform larvae. These second generation adults conformed morphologically and physiologically to those of the parent generation.

Unfertilized free-living females, developed on cultures without males, failed to produce viable embryos. It is, therefore, believed that parthenogenesis in the free-living phase does not exist.

In a later experiment 6 cultures produced second generation males and females which, in turn, produced third generation filariform larvae. On 3 of these cultures males as well as filariform larvae of the third generation developed and on one of these, 2 third generation females were present in addition to a male and filariform larvae. Eggs of the fourth generation were found on the sixth and last day. A culture with first, second and third generation sexual forms and larvae of all 3 generations, was fixed with hot 2% formalin and all worms were counted and classified according to the stage of development. There had been planted 9 *S. simiae* eggs which hatched and developed into 4 males, 4 females and 1 first generation filariform larva. On the third day, second generation rhabditiform larvae were present in abundance. The first generation males were dead on the fourth day but the 4 females were still alive and in addition there were 10 males and 6 females of the second generation, the latter laying third generation eggs. These eggs developed into males and rhabditiform and filariform larvae of the third generation. The count demonstrated the presence of 4 eggs, 53 rhabditiform larvae, 22 males, 6 females and 553 filariform larvae, a total of 638. Deducting the parent generation represented by 4 males, 4 females and 1 filariform larva there remain 629 descendants. This does not take into consideration the eggs and larvae which died within the first few days and had disintegrated beyond recognition. As only 6 females, the number corresponding to those of the second generation, were found at the final count, it is believed that the females of the first generation had died and disappeared entirely. This must have been true for the original males as well, since it was known from previous culture work and controls of the experiment described that they consistently failed to survive as long as the females.

The recovery of over 600 worms could not possibly be accounted for without the intercalation of a second generation of sexual forms, since it was known from previous *in vitro* culture work with this species that the maximum number of offspring per female was 80 and the average 60. Thus 4 first generation females could not conceivably produce more than 320 and probably would not produce over 240 offspring. Considering 60 offspring as an average per female then one would expect 600 from the 4 females of the first generation and the 6 females of the second generation, a figure closely corresponding to the number actually recovered.

It is believed that this continued propagation as demonstrated on culture media occurs under suitable natural conditions. As far as is known this is the first demonstration of continued propagation of the free-living phase of any species of *Strongyloides.*

### 8146 C

## Absorption of Drugs Through the Oral Mucosa. II.

ROBERT P. WALTON.

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The relative effectiveness of certain drugs by the sublingual method of administration has been previously reported.[1] In this earlier communication, approximate ratios were determined for effective sublingual doses and the similarly effective subcutaneous doses. In the present report, a similar ratio for 4 other drugs is described. These latter figures were determined principally for the purpose of studying the correlation between oral absorbability and certain fat-water solubility relationships. The general method of experimentation was the same as previously described. All drugs were used in the form of the hydrochloride salt and the dosage expressed on this basis.

*Cocaine.* Using excitement effects as the criterion of absorption, the sublingual/subcutaneous ratio in dogs was approximately 2 to 1. Twelve experiments were carried out with sublingual doses ranging from 15 mg./kg. to 50 mg./kg. These experiments included 3 trials with esophagotomized dogs in which a 5% tannic acid solution and suction drainage was used to prevent absorption from cut surfaces. The most satisfactory results were obtained following the intravenous injection of 1 mg. atropine sulphate to paralyze salivary secretions. Four trials were made with subcutaneous doses ranging from 15 to 30 mg./kg. These results agreed well with the more extensive observations described by Eddy[2] and Sollmann and Hanzlik.[3]

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[1] Walton and Lacey, *J. Pharm. and Exp. Therap.*, 1935, **54**, 61.

*Thebaine* (Paramorphine). Using reflex hyperexcitability in dogs as the criterion of effect, the sublingual/subcutaneous ratio was found to be greater than 4 to 1. Ten mg./kg. subcutaneously gave definite effects of increased reflex hyperexcitability which passed off in 120 minutes. Such effects were obtained at dosages which did not affect the Thiry fistula tracing. Five trials were made with sublingual dosages of 15 to 30 mg./kg. and 5 trials were made with subcutaneous doses of 5 to 10 mg./kg. The drug was of some theoretical interest because its chemical structure suggested a favorable fat-water solubility relationship.

*Emetine.* Using emesis as a criterion of effect, the sublingual/subcutaneous ratio in dogs was found to be greater than 6 to 1. With sublingual doses from 6 to 36 mg./kg. no vomiting occurred except in one case where there was considerable swallowing (5 trials, 2 with the maximum dose). Subcutaneous doses of 6 mg./kg. caused vomiting in each of 3 trials. These latter results are in good agreement with the more extensive observations of Eggleston and Hatcher with intravenous doses.[4]

*Diacetylmorphine.* Using the motor stimulant effect on Thiry fistulae which is characteristic of morphine derivatives, the sublingual/subcutaneous ratio was approximately 3 to 1. The results given in Table I were obtained according to a procedure previously described.[5] The expression of results by means of + symbols is modified here by the use of fractional values for these same symbols. It is considered that this is in keeping with the order of accuracy of this method of expression.

The subcutaneous effects are in agreement with the statement of

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[2] Downs and Eddy, *J. Pharm. and Exp. Therap.*, 1932, **46**, 195.

[3] Sollmann and Hanzlik, An Introduction to Experimental Pharmacology, 1928, 279.

[4] Eggleston and Hatcher, *J. Pharm. and Exp. Therap.*, 1915, **7**, 233.

[5] Walton and Lacey, *J. Pharm. and Exp. Therap.*, 1935, **54**, 53.

TABLE I.
Effects Produced by Diacetylmorphine on Motor Activity of Thiry Gut.

| Subcutaneous |  |  | Sublingual |  |  |
|---|---|---|---|---|---|
| Dose mg./kg. | Effect | Time for Max. Effect, Min. | Dose mg./kg. | Effect | Time for Max. Effect, Min. |
| 0.10 | 2.0+ | 1.5 | 0.20 | 1.8+ | 4.0 |
| 0.10 | 2.7+ | 1.5 | 0.40 | 2.6+ | 4.0 |
| 0.20 | 3.7+ | .7 | 0.50 | 3.6+ | 6.5 |
| 0.40 | 4.5+ | 1.5 | 0.50 | 4.0+ | 5.0 |
| 0.40 | 4.6+ | 1.0 | 1.00 | 3.6+ | 7.0 |
| 0.60 | 10.0+ | 1.0 |  |  |  |
| 1.00 | 5.2+ | 1.0 |  |  |  |
| 3.00 | 13.5+ | 1.5 |  |  |  |

Plant and Miller[6] that such effects are similar to those produced by morphine. Three sublingual trials with an esophagotomized dog gave prompt spastic effects in the dosage range used above. These effects could not be evaluated, however, because the control periods were irregular. Eight trials with human subjects in dosages ranging from 12 to 35 mg. showed no clear effects, except in 1 of 3 trials at this maximum dosage. The absorption is evidently not sufficient for this method to serve as a practical method of administration.

*Summary.* The ratios of sublingual doses to similarly effective subcutaneous doses have been described for 4 drugs. These approximate ratios are as follows: Cocaine, 2; diacetylmorphine, 3; thebaine, greater than 4; emetine, greater than 6.

## 8147 C

## Absorption of Drugs Through the Oral Mucosa. III. Fat-Water Solubility Coefficient of Alkaloids.

ROBERT P. WALTON.

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It has been shown that the absorption of drugs from the mouth is strictly a selective process.[1, 2] With even a closely related group of drugs, such as the common alkaloids, marked differences are exhibited by the individual members. Apomorphine, for instance, can be effectively administered by simple sublingual application, whereas morphine is very poorly absorbed. The diacetyl derivative of morphine is absorbed fairly well, whereas the monomethyl derivative is absorbed very poorly. Since all 4 of these drugs are closely related both chemically and pharmacologically, the explanation for this phenomenon of selectivity might reasonably be expected to depend on physical differences. The most prominent suggestion is that of a correlation with the oil-water distribution coefficient. This relationship has been extensively developed for other problems of cell permeability,[3, 4] and also has been described as being the decisive factor in absorption through the oral mucosa.[5] However, for this latter aspect of the correlation, the only previous demonstration of the principle is the fact that glycerol trinitrate is readily soluble in oils and only sparingly soluble in water. The present report presents a study of this relationship for a series of common drugs which have more limited oil solubilities.

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[6] Plant and Miller, *J. Pharm. and Exp. Therap.*, 1923, **21**, 202.

[1] Walton and Lacey, *J. Pharm. and Exp. Therap.*, 1935, **54**, 61

[2] Walton, R. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1486.

In Table I the ratio of sublingual doses to similarly effective subcutaneous doses is compared with the fat-water solubility coefficient of the same drug. The sublingual/subcutaneous ratios, which have been previously described[1, 2] are not to be considered as natural constants in the same sense as solubility figures, but simply as approximate expressions of their absorption behavior. The oil-water solubility coefficients are usually considered equivalent to the distribution ratios of the same drugs in a 2-phase system made up of equal volumes of oil and water.[6] All the figures for oil solubility and one of those for water solubility (dilaudid) were determined for the purposes of this correlation. With the exception of the oil solubility of cocaine these data were not available in the literature. The figures for water solubilities, with the one exception were obtained from the literature. Since the 2 sources of data, the U. S. Dispensatory and a special study by Kolthoff,[7] are based on different conditions, both are quoted in separate columns of the table. The figures reported by Kolthoff cannot be compared directly with those in the U. S. Disp. because of a difference in the temperatures of determination. Special interest may be attached to the results

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[3] Meyer and Gottlieb (Meyer and Pick), Die experimentelle Pharmakologie, 1933.

[4] Beutner, Physical Chemistry of Living Tissues and Life Processes, 1933.

[5] Meyer and Gottlieb, *loc. cit.*, 6.

[6] Hill, A. E., in A Treatise on Physical Chemistry, edited by H. S. Taylor, 1930, **1**, 477.

[7] Kolthoff, *Biochem. Z.*, 1925, **162**, 289.

obtained by Kolthoff because of the unique titration method involved in their determination. According to the U. S. Disp., the solubility of cocaine in olive oil is 80 mg. per cc.

*Method for Determination of Solubility in Olive Oil.* The general procedure was to obtain a saturated oil solution of the alkaloid at 25°, to extract a definite volume of this saturated solution with aqueous acid and to determine the quantity of the alkaloid in the acid extract by precipitating with phosphotungstic acid. Another procedure, used for ascertaining the approximate order of solubility, consisted in stirring weighed quantities of the alkaloids with successively increasing volumes of oil until clear solutions were obtained. In both these procedures, the solutions were stirred with a series of motor-driven stirring shafts operating at 1,000-1,500 R.P.M. All alkaloids except apomorphine were oven-dried at temperatures of 90-115°.

According to the first procedure, all mixtures were stirred 300 minutes or more although approximate saturation was obtained in 100 minutes for those cases in which this point was determined. Suspended solids were removed from the saturated oil solutions by centrifuging and the clear solutions were extracted by shaking with M/10 HCl in amounts and instalments depending on the concentration of the various alkaloids. The volumes were so adjusted as to have about 1 mg./cc. of alkaloid in the total acid extract. The alkaloids were precipitated from the extract with an excess of phosphotungstic acid made up as follows: Sodium tungstate 100 gm., disodium phosphate 75 gm., water 500 cc., and con. nitric acid 70 cc. After centrifuging in calibrated tubes, the volumes of the precipitates were estimated by matching with precipitates of equal volume obtained by dissolving weighed amounts of the same alkaloid in M/10 HCl and precipitating and centrifuging at the same time. Each determination of concentration in the extracts represented several series of such precipitations. The whole performance was carried out 2 to 4 times for each drug.

In the second procedure mentioned above, weighed quantities of dried alkaloids were stirred with increasing volumes of oil until the solutions became clear, 150 minutes of stirring being considered sufficient for equilibrium with each successive volume of oil. Clear solutions were obtained with cocaine, strychnine, atropine, and heroin and the results corresponded fairly well with those obtained by the other procedure. With the other drugs indicative results were obtained but the operations were not carried to complete solution. For instance, morphine and dilaudid proved very insoluble.

Codeine dissolved to give a fairly clear solution which became cloudy on further stirring. Possibly the codeine underwent decomposition to the more insoluble morphine.

The order of accuracy of the oil solubility determinations was considered sufficient for the purposes involved. Apomorphine presented a special problem because of its highly unstable character. A quantity of the alkaloid was obtained in a sealed ampoule (through a special courtesy of Merck and Co.) and ordinary precautions taken to prevent its oxidation. The water solubility of dihydromorphinone (dilaudid) was determined by stirring an excess of alkaloid with boiled, distilled water and estimating the concentration by precipitation with phosphotungstic acid as before.

The oil-water distribution coefficient of nicotine was determined at 40° by stirring 5% solutions in water with equal volumes of oil and 5% solutions in oil with equal volumes of water. The nicotine in the aqueous layers was determined by titration. The average coefficient thus obtained by 3 trials was 2.6 and is lower than would be expected from its relatively high oral absorption.[8, 9] This divergence may be due to the highly irritant nature of the alkaloid or to its extreme oil solubility (complete miscibility).

TABLE I.

| Drug | Oil solubility mg./cc. 25° C. | Water solubility, mg./cc. (Kolthoff) 15° C. | Water solubility, mg./cc. (U. S. Disp.) 25° C. | Oil-water sol. coeff. (Kolthoff) | Oil-water sol. coeff. (U. S. Disp.) | Ratio sublingual to subcu. dose |
|---|---|---|---|---|---|---|
| Cocaine | 45.0 | 1.2 | 1.6 | 37 | 28 | 2 |
| Apomorphine | 1.8 | 0.09 | — | 20 | — | 2 |
| Diacetylmorphine | 10.0 | — | 0.6 | — | 17 | 3 |
| Strychnine | 3.2 | 0.09 | 0.15 | 35 | 21 | 4 |
| Thebaine | 8.0 | 0.7 | — | 12 | — | 4+ |
| Emetine | 10.0 | 1.1 | — | 9 | — | 6+ |
| Atropine | 14.0 | 1.6 | 2.1 | 9 | 7 | 8 |
| Morphine | 0.05 | 0.15 | 0.3 | 0.3 | 0.15 | 10 |
| Dihydromorphinone | 0.4 | — | (2.0) | — | 0.20 | 15 |
| Codeine | 16.0 | — | 8.0 | — | 2.0 | 15+ |

Although the correlation shown in Table I is by no means regular, the nature of the data involved is such that no high degree of regularity can be expected. The feature which is clearly demonstrated by the table is that certain drugs which are poorly absorbed have relatively low oil-water solubility coefficients and closely related drugs which are well absorbed have relatively high coefficients.

It may be concluded that the oil-water solubility coefficient is an

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[8] Franke and Thomas, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1177.

[9] Franke and Thomas, *J. Pharm. and Exp. Therap.*, 1933, **48**, 199.

important factor in the selective oral absorption of these drugs. Other factors which may be significant are relative potency, degree of local vasoconstriction, irritation and alkalinity or acidity. It is also probable that absorption is favored by high oil solubility independent of the oil-water distribution coefficient, as is the case with nicotine.

### 8148 P

## Peripheral Course of Pain-Fibers Supplying Coronary Arteries and the Myocardium.

ROBERT M. MOORE AND ALBERT O. SINGLETON, JR.

*From the Department of Surgery, University of Texas, Medical Branch, Galveston.*

In decerebrate animals intra-arterial injection of an irritating solution results in stimulation of pain-elements associated with the small blood-vessels. The stimulation is evidenced by reflex activity of a pseudoaffective nature (Moore and Porter[1]). On injection of a coronary artery the reflex effects include arching of the back, movements of the extremities, snapping of the jaws, tossing of the head, and increased respiratory excursions. Attempts to prevent such activity by surgical neurectomy have provided information regarding the course of the pain-fibers concerned.

The experiments were performed upon cats. After guillotine decerebration, etherization was discontinued, the thorax was entered under artificial respiration, the pericardium opened and a thread passed beneath the stem of one or other coronary artery proximal to its bifurcation. Injection of 0.75 cc. 20% lactic acid distal to the thread caused instantaneous and very marked reflex activity associated with a rapidly progressing, black infarction of practically the entire wall of that half of the heart (Fig. 1). The injection was followed in 5 or 10 seconds by acute dilatation of both chambers.

Injection of the left coronary artery caused immediate and marked reflex activity in each of 17 animals in which the left thoracic sympathetic trunk was intact, although these animals had been subjected to unilateral or bilateral cervical sympathectomy or vagotomy. In contrast were the results in 10 animals in which the left thoracic sympathetic trunk had been resected from above the stellate ganglion to the 7th rib. Although the cervical trunks and the right

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[1] Moore, R. M., and Porter, E. L., *Am. J. Physiol.*, 1934, **109**, 76.

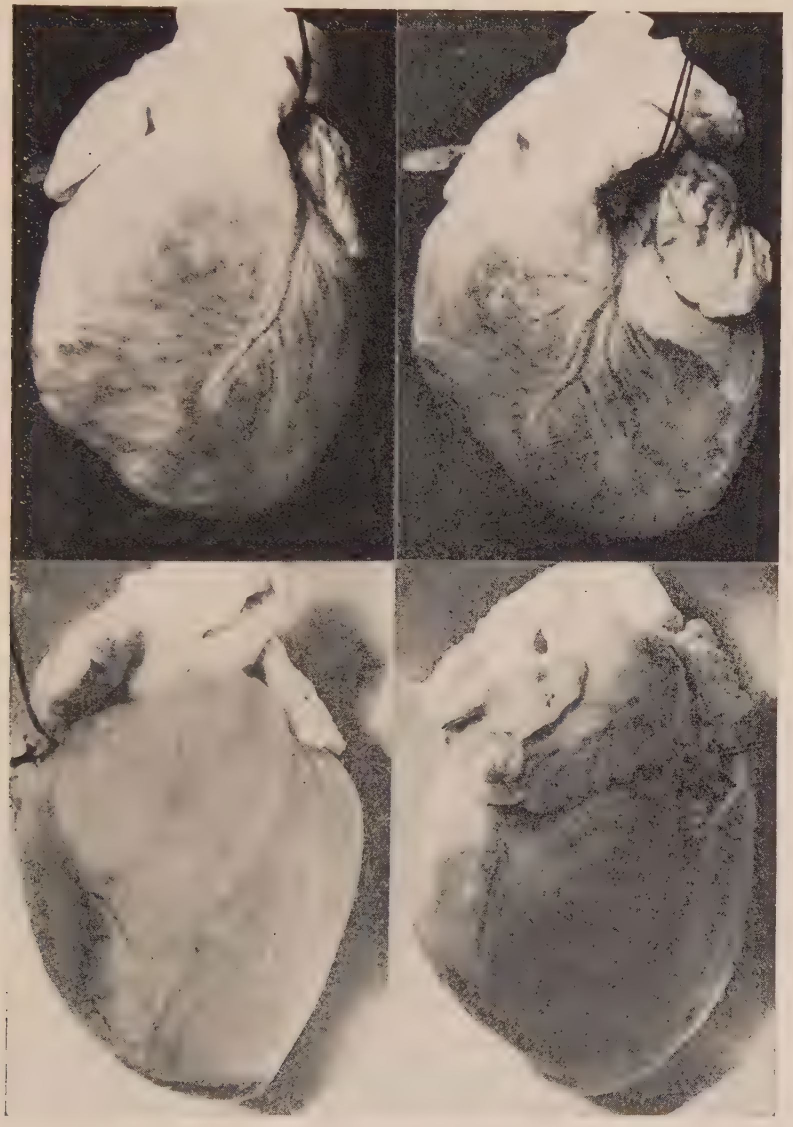

FIG. 1.

Photographs of hearts following injection of acid into the coronary artery in the decerebrate animal. The blackened areas represent the infarctions. Anterior and left lateral views after injection of the left coronary artery are shown above. Below are anterior and posterior views following injection of the right artery. In either instance stimulation of pain-elements can be prevented by resection of the *ipsilateral* upper thoracic sympathetic chain.

thoracic trunk were not disturbed, in none of these animals did visible reflex effects occur until ventricular dilatation had become extreme and there was advanced asphyxia.

Injection of the right coronary artery caused no visible reflex effects in 4 animals in which only the right cervicothoracic sympathetic trunk had been resected. Similarly, no reflex reaction was obtained in 4 of 5 animals in which the right upper thoracic trunk had been resected and the right cervical trunk left intact.

It is apparent, then, that the pain-fibers ending in the region of either coronary artery reach the spinal cord by way of the *ipsilateral* sympathetic chain. Furthermore, the great majority of these fibers must pass from the heart to the upper thoracic segments of that chain.

If this scheme of afferent innervation of the coronary arteries also applies in man, resection or injection of the upper thoracic portion of the *ipsilateral* sympathetic trunk should be the operation of choice for the relief of coronary pain. Moreover, the results suggest that reference of coronary pain to either upper extremity indicates stimulation of pain-endings associated with the coronary vessels of the corresponding side.

We wish to express indebtedness to Dr. J. F. Pilcher, who made the photographs.

### 8149 P

## Relative Protective Value of Alkaline Duodenal Juices Against Gastrojejunal Ulceration.

MICHAEL DEBAKEY. (Introduced by A. Ochsner.)

*From the Department of Surgery, Tulane University School of Medicine, New Orleans, La.*

A review of the voluminous literature which has now accumulated on the relative protective value of the alkaline duodenal juices in preventing ulcer formation reveals a bewildering maze of confusion. Much of this diversity of opinions and contradictory experimental results has been due in great measure to the observations and comparisons made by different investigators under varied and diverse circumstances. Because of the existing difficulty in drawing conclusions from the confusing and contradictory experimental results, this investigation was made.

The investigation was divided into 4 groups of experiments: (1) Group 1—Severance of the pylorus and closure of both the gastric and duodenal ends and the reestablishment of continuity of the gastro-intestinal tract by gastrojejunostomy; (2) Group 2—Exactly the same procedure as in Group 1 but with the addition of transplantation of the common bile duct into the terminal ileum; (3) Group 3—Exactly the same procedure as was performed in the first group but with the addition of ligation and division of the accessory pancreatic (whenever it was found) and transplantation of main pancreatic duct into the terminal ileum; (4) Group 4—Exactly the same procedure as in Group 1 but with the addition of transplantation of both bile and pancreatic ducts into terminal ileum. Obviously all the groups are similar with the exception of one factor, the deviation of bile in one group, the deviation of pancreatic juice in another, and the deviation of both bile and pancreatic juice in the last group. Thus, because of the comparability of the 4 groups of experiments, any difference in results obtained can be accounted for by the one different factor characterizing each group.

A total of 60 dogs was used in the experiment—20 in the first group, 20 in the second, 10 in the third, and 10 in the fourth group. In Group 1, 50% of animals living from 9 to 271 days postoperatively developed typical subacute and chronic ulcers. These ulcers were grossly and microscopically similar to those occurring in man. All the ulcers developed at a characteristic site; *i. e.*, in the jejunal mucosa of the efferent loop. The probable explanations for the high incidence of ulcer formation after this procedure and their characteristic location are that the jejunal mucosa has little resistance to the acid gastric chyme, and the efferent loop of the jejunum is bathed with gastric chyme of relatively higher acidity than the afferent loop or the jejunal mucosa immediately opposite the stoma.

In Group 2, 90% of the animals living from 7 to 60 days postoperatively developed typical subacute and chronic ulcers. The 40% higher incidence of ulcer formation in this group than in Group 1 is probably due to the absence of bile in the alkaline duodenal secretions in the former group. Other differences in this group are: (1) the occurrence of ulcers in the anastomotic suture line in four instances with pieces of black silk hanging free from the base in 2, and (2) the occurrence of ulcers in the jejunal mucosa immediately opposite the stoma in 6 animals. The high incidence of these ulcers (anastomotic, 22.2% and opposite stoma, 33.3%) proximal to the usual site in the efferent loop emphasizes more strongly the acid neutralizing influence of bile.

In Group 3, 70% of the animals living from 7 to 56 days postoperatively developed typical subacute and chronic ulcers. There were 2 ulcers in the anastomotic suture line, one ulcer in the jejunal mucosa opposite the stoma, and one ulcer in the afferent loop. The 20% higher incidence of ulcer formation in this group as compared with Group 1 is probably due to the absence of the alkaline pancreatic juice in the former. The fact that in Group 2 there was a 20% higher ulcer incidence than in Group 3 and that ulcers occurred with greater frequency in the more unusual sites (anastomotic

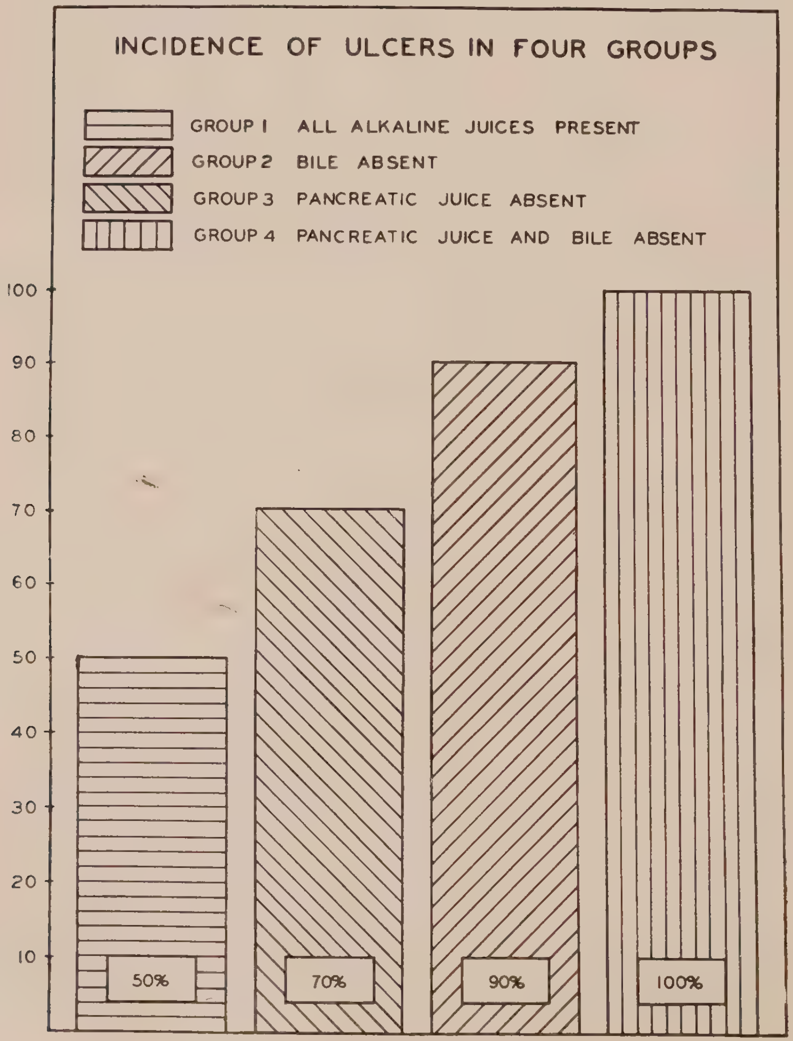

FIG. 1.

suture line and in jejunum opposite stoma) in the former indicates that bile probably exerts a greater acid neutralizing power than pancreatic juice.

In Group 4 all of the animals (100%) living from 12 to 51 days postoperatively developed typical subacute and chronic jejunal ulcers. As both the bile and pancreatic juices were absent and only the succus entericus remained to protect the jejunal mucosa, this 100% incidence of ulcer formation indicates that the latter alkaline juice has little or no protective influence in the prevention of jejunal ulcers.

A comparison of the results obtained in the 4 groups of experiments is illustrated graphically in Fig. 1. These results seem to justify the following conclusions: (1) Of all the constituents of the alkaline duodenal juices bile has the most significant and effective influence in preventing the formation of jejunal ulcers; (2) the pancreatic juice is next most protective; and (3) the duodenal secretion, the succus entericus, has little or no protective influence in the prevention of jejunal ulcers.

# Minnesota Section

*University of Minnesota, May 23, 1935.*

### 8150 C

## Effect of X-Rays on Methylene Blue and on Trimethyl Thionine.

WILHELM STENSTRÖM AND HAROLD R. STREET.

*From the Division of Biophysics, Department of Physiology, University of Minnesota.*

When a dilute aqueous solution of methylene blue is exposed to X-rays or ultra-violet radiation, a color change is effected. This change has been utilized to determine the relative intensity of the radiation.[1, 2] It is, however, difficult to obtain consistent results with solutions made up at different times. One reason, therefore, is undoubtedly to be found in the fact that methylene blue contains small amounts of related substances which also have strong colors. The most conspicuous of these substances is trimethyl thionine, which is present as an impurity in varying amounts up to 10% in methylene blue.[3] This study was undertaken mainly to find out how the presence of trimethyl thionine may affect the color change of methylene blue.

Trimethyl thionine was synthesized according to Fierz-David's[4] method for preparing methylene blue with the substitution of monomethyl aniline for the dimethyl aniline called for in Fierz-David's directions. There is no possibility of the presence of methylene blue in this product. The methylene blue was recrystallized several times. However, it is known that the purest methylene blue available contains a certain amount of trimethyl thionine.

The absorption curves of unirradiated methylene blue and trimethyl thionine were determined by means of a spectro-photometer.

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[1] Stenström, Wilhelm, and Lohmann, Anne, *Radiology*, 1933, **21**, 29; *ibid.*, 1934, **22**, 304.

[2] Webster, T. A., Hill, L., and Eidinow, A., *Lancet*, 1924, **1**, 745.

[3] Holmes, W. C., *Stain Technology*, 1928, **3**, 45.

[4] Fierz-David, The Fundamental Processes of Dye Chemistry, J. & A. Churchill, London, 1921, p. 174.

These curves shown in the figure are very similar, but have distinct differences. The absorption of methylene blue has a peak between 6650 Å. U. and 6700 Å. U. and extends further into the red than that of trimethyl thionine which has its peak between 6500 Å. U. and 6550 Å. U.

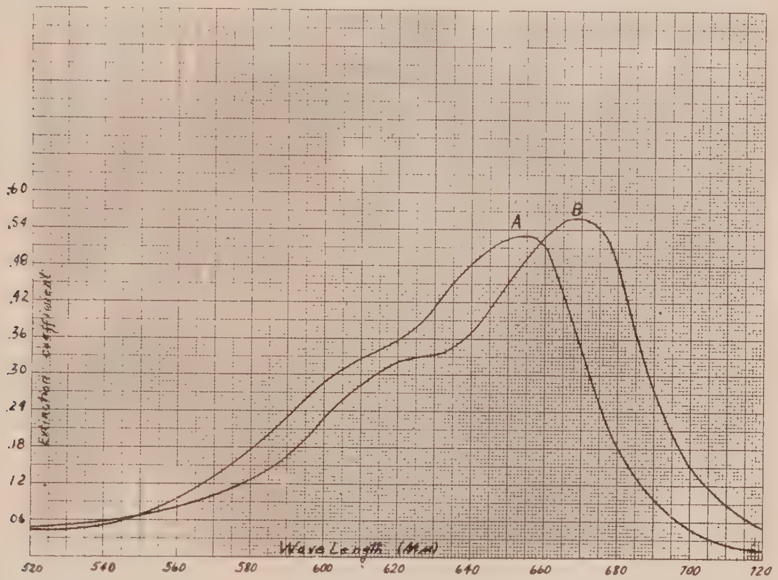

FIG. 1.

Absorption curves of methylene blue and trimethyl thionine, concentration 3 mg. per liter. (A) trimethyl thionine, (B) methylene blue.

Since in former work the change in methylene blue concentration was measured by the change in extinction coefficient at 6700 Å. U., solutions of methylene blue and trimethyl thionine were prepared which had the same extinction coefficient at 6700 Å. U. A solution of methylene blue with a concentration of 3.26 mg. per liter was used. In order to have the same absorption it was necessary to use a concentration of trimethyl thionine of 5.25 mg. per liter. When these solutions were exposed in small glass tubes to the same amount of X-rays (2460 roentgens) the absorption at different wavelengths was changed to a relatively different extent for the 2 solutions. Thus methylene blue had its maximum reduction of 21.7% at 6700 Å. U. where trimethyl thionine had a much smaller change, 12.4%. The maximum change of absorption for trimethyl thionine was at 6500 Å. U. with 14.6% reduction, at which wavelength methylene blue showed 17.5% reduction. Since in using methylene blue as indicator for X-ray intensity we have previously determined the reduction of absorption at 6700 Å. U. it seems that the change at this wavelength will be smaller the greater the amount of trimethyl thionine present as an impurity. With a mixture of 10% trimethyl thionine and 90% methylene blue the change at 6700 Å. U. ought to be about 21% instead of 22%. In one experiment of this type no definite difference was discovered; however, this was not surprising as the difference of 1% is of the order of magnitude of the experimental error. It seems in any case that the presence of trimethyl thionine should reduce the sensitivity of the methylene blue solution to a certain extent though not enough to account for all the difference we have noticed with solutions made up at different times or from different samples of methylene blue.

## 8151 C

## Requirement of the Flour Beetle (Tribolium confusum Duval) for Vitamins in the B Group.

HAROLD R. STREET AND LEROY S. PALMER.

*From the Division of Biochemistry, University of Minnesota, St. Paul.*

Insects offer certain possibilities for vitamin assay. If the vitamin requirements of certain insects were known it should be possible to assay vitamins with much less time and labor than with rats or similar animals. A suitable insect for nutritional research is the flour beetle, *Tribolium confusum* Duval.

When raised on whole wheat flour at 32° and 70% humidity, the larvae transform to the pupal stage in an average time of 16 days. Sweetman and Palmer[1] found that on a diet of purified food materials devoid of all vitamins the insects did not pupate. The addition of a small amount of material containing the vitamin B complex enabled the larvae to pupate in a normal time.

The renewed interest in methods for assay of the vitamins in B group brought about by the recent advances in the chemistry of these substances prompts us to report briefly experiments conducted by us in 1931-32. In this study an attempt was made to determine which members of the vitamin B complex are necessary for Tribolium. A basal diet of casein 15, crisco 3, Osborne-Mendel salt mixture 4, dextrin to 100 was used. Various vitamin supplements were added to 5 gm. quantities of the basal diet, and the food was placed in a vial with 20 two-hour-old larvae. The vials were kept in a temperature cabinet at 32°C. and 70% humidity and the average time of pupation recorded. A vitamin $B_1$ concentrate prepared from rice polishings was used. The average time required for pupation with the various supplements to the basal diet were: vitamin $B_1$ concentrate equivalent to 50% rice polish no pupation, 5% autoclaved yeast no pupation, 1% autoclaved yeast plus vitamin $B_1$ concentrate equivalent to 2% rice polish 19.2 days. This indicates that Tribolium requires a heat stable factor in addition to vitamin $B_1$. It was found that this heat stable factor was destroyed by autoclaving at pH 13 for 4 hours.

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[1] Sweetman, M. D., and Palmer, L. S., *J. Biol. Chem.*, 1928, **77**, 33.

With 1% autoclaved yeast to supply the heat stable factor the larvae responded in a quantitative manner to the addition of the vitamin $B_1$ contained in small amounts of rice polish as follows: with 0.1% rice polish average time of pupation was 20.3 days; 0.05% rice polish, 23.8 days; 0.025% rice polish, 40.2 days. With 1% rice polish as the sole addition to the basal diet the average time of pupation was 98 days.

### 8152 C

## Positive Flocculation Tests in Rabbits Inoculated with Flocculate from Human Syphilitic Serum.

F. RYTZ. (Introduced by W. P. Larson.)

*From the Clinical Laboratories of the Minneapolis General Hospital, Minneapolis, Minnesota.*

In an effort to determine the nature of flocculate obtained from human syphilitic serum, by means of flocculation tests for syphilis, an attempt was first made to produce precipitins in rabbits by intravenous inoculations with such flocculate. Attempts in dissolving the flocculate were at the time unsuccessful. A rabbit was therefore inoculated with flocculate suspended in sterile saline. After 5 intravenous injections the rabbit serum was tested for precipitin formation and found negative in various dilutions. On the other hand, the serum from that rabbit, when added to 2 plus positive syphilitic serum, changed the positiveness to a strong 4 plus reaction.

An attempt was then made to produce in rabbits, by inoculation of flocculate from human syphilitic serum, the antibodies causing flocculation of antigen in syphilitic serum.

In this experiment 16 albino rabbits were used, 10 males and 6 females. The flocculation test employed was that originated in the laboratories of the Minneapolis General Hospital.[1] This was supplemented by the Wassermann reaction as an extra check and as a special precaution. The latter test was performed by a technician who had no knowledge of the source of the blood samples involved. After the rabbits proved to have negative flocculation tests, they were inoculated intravenously with from 16 to 60 units of flocculate from the M.G.H. test or from the Kahn test. The flocculate was obtained from human syphilitic serum which proved to be 4 plus positive by M.G.H., Kahn and Wassermann methods. The flocculate from one 4 plus reaction was called 4 units. Before injection, the flocculate was centrifuged and washed 3 times in sterile saline. The dose was finally suspended and shaken in 5 cc. of sterile saline. At intervals of 5 days the animals were thus injected intravenously, with increasing doses, from 2 to 5 times. The last 2 injections consisted, except in the case of 3 of the animals, of 120 units of flocculate each.

After 2 to 3 inoculations all of the animals showed positive flocculation tests, varying in positiveness from 2 to 4 plus. In most of the animals 4 inoculations resulted in strong 4 plus reactions, and 4 animals gave reactions from 9 plus to 20 plus by quantitative method. In most of the cases the final test was performed 8 days after the last injection.

Two control animals were injected, in the same manner and in the same proportion as the test animals, with antigen emulsion alone. The serum from these control animals remained negative throughout the experiment.

Of the 14 rabbits inoculated with flocculate, 11 were given flocculate from our own test, and 2 received flocculate from the Kahn test. No difference was noted in potency of the flocculate from the 2 tests.

One animal, rabbit No. 4, was injected with flocculate from the serum of rabbits No. 1 and No. 3. After 3 intravenous injections its serum was 3 plus positive. The fourth injection, 100 units of flocculate, was given under ether anesthesia. A week later the positiveness of that rabbit had dropped from 3 plus to 1 plus. It was then given another injection of 100 units of flocculate from the serum of rabbit No. 3. Eight days later the positiveness had risen

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[1] Rytz, F., *Am. J. Clin. Path.*, 1935, **5**, 159.

TABLE I.

No. of flocculate units injected and resulting positiveness of each rabbit. The serum unit for the flocculation test is 0.15 cc. The quantitative unit is the amount necessary for a 4 plus reaction.

| Rabbit No. | No. of injections | Total No. of floc. units | Flocculat. reaction | Wassermann reaction | Remarks |
|---|---|---|---|---|---|
| 1 | 5 | 408 | 20+ | 4+ | One-fifth unit of serum gave a 4-plus reaction. Showed strongly 4-plus flocc. reaction 2 months after the last injection. |
| 2 | 2 | 48 | 2+ | neg. | Developed snuffles. Died. |
| 3 | 3 | 136 | 4+ | 4+ | ,, ,, ,, |
| 4 | 5 | 344 | 3+ | neg. | Injected with flocculate from rabbits 1 and 3. Ether anesthesia reduced the positiveness to 1-plus. Positiveness returned later to 2-plus. |
| 5 | 4 | 360 | 4+ | 4+ | Positiveness 2-plus 3 days after ether anesthesia. |
| 6 | 4 | 360 | 4+ | 4+ | |
| 7 | 4 | 360 | 3+ | 2+ | |
| 8 | 4 | 360 | 3+ | 4+ | |
| 9 | 4 | 360 | 16+ | 4+ | Injected with flocculate from the Kahn test. One-fourth unit of serum gave a 4-plus flocc. reaction. |
| 10 | 4 | 360 | 4+ | 4+ | Injected with flocculate from the Kahn test. |
| 11 | 4 | 360 | 9+ | 4+ | One-third unit of serum gave 3-plus flocc. reaction. |
| 12 | 4 | 360 | 4+ | 4+ | |
| 13 | 4 | 360 | 12+ | 4+ | One-fourth unit of serum gave 3-plus flocc. reaction. |
| 14 | 4 | 360 | 3+ | 1+ | |
| 15 | 4 antig. emuls. | | neg. | neg. | Injected with 4.5 cc. of antigen emulsion only. |
| 16 | 4 ,, ,, | | ,, | ,, | ,, ,, ,, ,, ,, ,, ,, ,, |

from 1 plus to 2 plus. Two months later the serum of this rabbit was still 2 plus positive.

Rabbit No. 5, after showing a 4 plus reaction, was also anesthetized and kept under ether anesthesia for 5 minutes. Three days later the positiveness of its serum had dropped from 4 plus to 2 plus. No further injections were given to that rabbit.

After rabbit No. 9 had given a final reaction of 16 plus, it was killed by cutting the jugular vein. Fifteen cc. of clear serum was thus obtained which yielded 1,620 units of flocculate. The serum, therefore, contained 1,260 units of flocculate above the original 360 units injected.

*Conclusions.* (1) For lack of a better name, the antibodies causing flocculation of antigen in human syphilitic serum might be termed "flocculins". (2) It has been shown that flocculate obtained from human syphilitic serum, by means of flocculation tests for syphilis, will cause positive flocculation tests in rabbits when inoculated intravenously. Such reactions will remain positive for at least 2 months after the last injection. Rabbits inoculated with antigen emulsion alone do not develop positive flocculation tests. (3) Apparently, the serological factors involved in the formation of "flocculins" in the animal body are influenced by ether anesthesia. (4) Flocculate obtained from the serum of a rabbit injected with flocculate from human syphilitic serum, will cause the formation of "flocculins" in another rabbit when injected intravenously. (5) The serum of a rabbit inoculated with a definite number of flocculate units, may develop many more units than were thus injected.

### 8153 C

## Intra-Enteric Pressure in Experimental and Clinical Intestinal Obstruction.*

LOUIS SPERLING, JOHN R. PAINE, and OWEN H. WANGENSTEEN.

*From the Department of Surgery, University of Minnesota.*

As a preliminary to a series of experiments on the effect of increased intra-enteric pressure upon the physiology and structure of the bowel wall, occasion was taken to measure the levels of sustained pressure which obtained in the intestine of dogs with low ileal obstruction.

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* This work was supportd by the Medical Research Fund of the Graduate School, University of Minnesota.

The intra-enteric pressure was determined in 19 dogs with low ileal type of obstruction of one to 7 days' duration. Simple obstruction was produced by sectioning the terminal ileum and inverting both ends. At the stated duration of obstruction, the animals were anesthetized, the abdomen opened, and a large calibre needle connected to a water manometer was inserted into the lumen of the obstructed ileum. Pressure readings ranged from 4 to 19 cm. of water, or roughly 4 times the normal. Active peristalsis was always observed even after 7 days of obstruction. The intra-enteric pressure in this series apparently increased with the duration of the obstruction. It was observed that the sustained pressure in low ileal obstruction was seldom above 14 cm. of water (Table I).

TABLE I.
Sustained Intra-enteric Pressure in Dogs with Simple Ileal Obstruction.

| Dog | Days Obst. | Pressure Cm. $H_2O$ | Dog | Days Obst. | Pressure Cm. $H_2O$ |
|---|---|---|---|---|---|
| 1 | 1 | 8 | 11 | 4 | 14 |
| 2 | 2 | 4 | 12 | 4 | 10 |
| 3 | 2 | 6 | 13 | 4 | 7 |
| 4 | 2 | 12 | 14 | 4 | 12 |
| 5 | 2 | 6 | 15 | 4 | 10 |
| 6 | 3 | 10 | 16 | 4 | 8 |
| 7 | 3 | 12 | 17 | 4 | 10 |
| 8 | 4 | 8 | 18 | 7 | 19 |
| 9 | 4 | 10 | 19 | 7 | 12 |
| 10 | 4 | 14 | | | |

On 6 additional dogs, 17 measurements of intra-enteric pressure were made at varying intervals after the establishment of obstruction in the following manner. A loop of gut of 3 to 4 inches in length was brought up on the abdominal wall and the adjacent skin was mobilized to cover it. When this satchel-like handle was well healed, complete obstruction was accomplished by inverting the ends of the severed terminal ileum. In a few instances 2 such subcutaneous loops were made in the same dog. The usual sustained intra-enteric pressure found varied between 6 and 8 cm. of water. The highest reading obtained was 17 cm. of water. These pressures are in accord with the findings of Owings and his associates.[1]

Opportunity has also been afforded to determine the intra-enteric pressures in 5 clinical cases of obstruction of the small bowel of mechanical origin. Pressures were determined at the time of operation (enterostomy) by connecting the enterostomy tube to a water manometer. The sustained pressure in these 5 cases varied between 4 and 18 cm. of water. With peristaltic activity, pressures of 20 to 30 cm. of water were noted.

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[1] Owings, J. C., *et al.*, *Arch. Surg.*, 1928, **17**, 507.

Pressures were obtained at operation when colostomy was performed for relief of distension in 8 cases of acute colonic obstruction due to malignancy. The sustained pressures varied in these 8 cases between 12 and 52 cm. of water; in 6 of the cases, pressures above 23 cm. of water were recorded, much higher figures than were observed in the small bowel. The duration of obstruction in these cases varied between 3 and 8 days. The significantly greater pressures in colonic obstructions are due to the action of the ileocaecal sphincter[2] which make of the obstructed colon a virtual closed-loop obstruction, in which the intra-enteric pressure may greatly exceed pressures observed in simple obstruction.[3, 4]

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## 8154 P

# A Crystalline Hydrobromide of Urobilin (Stercobilin)*

C. J. WATSON. (Introduced by H. A. Reimann.)

*From the Department of Medicine, University of Minnesota Hospital.*

The identity of crystalline urobilin and stercobilin, as isolated from human urine and feces, was established previously.[1-5] In confirmation of this the same crystalline hydrobromide has been prepared from each substance. The method of preparation is briefly as follows: The free urobilin is prepared from the hydrochloride as previously described.[2] It is not crystallized but is extracted from its chloroform solution with 25% hydrobromic acid. The latter is diluted with 3-4 volumes of distilled water, and the hydrobromide extracted with chloroform. The chloroform solution is dried over anhydrous $Na_2SO_4$, filtered, and concentrated to a small volume on the water bath. This is poured into at least 10 volumes of petro-

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[2] Sperling, Louis, *Arch. Surg.*, in press.

[3] Burget, G. E., *et al.*, *Arch. Surg.*, 1930, **21**, 829.

[4] Morton, J. J., and Sullivan, W. C., *Arch. Surg.*, 1930, **21**, 531.

*Aided by a grant from the University of Minnesota Graduate School.

[1] Watson, C. J., *Z. Physiol. Chem.*, 1932, **204**, 57.

[2] Watson, C. J., *Z. Physiol. Chem.*, 1932, **208**, 101.

[3] Watson, C. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1207.

[4] Watson, C. J., *Z. Physiol. Chem.*, 1933, **221**, 145.

[5] Watson, C. J., *J. Biol. Chem.*, 1933, **105**, 469.

leum ether. After precipitation of the hydrobromide, the precipitate is collected on a small filter paper, and dissolved by repeated extraction with small amounts of hot ethyl acetate. After moderate concentration, the solution is allowed to cool and crystallization soon commences. The yields have varied between 40-50%. The crystals have a very remarkable shape resembling long, narrow boats with several compartments (transverse cleavage lines). The substance is readily recrystallized from ethyl acetate, and has a light yellow color with slight orange cast. M.P. 145-150° (Shrinking above 135°). The absorption spectrum is the same as that of the hydrochloride, and the green fluorescence with zinc acetate appears to be fully as intense. It was conceivable that this substance was a degradation product of urobilin, not simply a hydrobromide. This possibility was excluded by ready conversion to the hydrochloride which was identified by the character of its crystals, and by preparation of its characteristic iron chloride molecular compound.[6]

Heilmeyer and Krebs[7] have recently stated that the hydrochloride of the urobilin which they isolated is a dihydrochloride. In this investigation stercobilin was also isolated by the method which they described. It was found to differ in no way from that isolated according to the method previously described by the writer.[5] From it the crystalline free substance, the iron chloride molecular compound, and the hydrobromide as described above were readily prepared. The hydrobromide contains 12% bromine, a value which agrees very well with formulae for a mono-hydrobromide. Further analyses of the hydrochloride indicate that if the chloroform is carefully removed prior to analysis, the values agree only with a formula for a mono-hydrochloride. In an earlier report the formula $C_{33}H_{44}N_4O_8$ was suggested for the crystalline-free stercobilin. Recent analyses of this, as well as of the hydrochloride and iron chloride molecular compound, which will be reported in detail in a separate publication, have shown that the oxygen content of the molecule is less than at first believed. This is also indicated by the analyses which Heilmeyer and Krebs have reported. Whether 6 or 7 oxygen atoms are contained in the molecule has not yet been determined. Recent analyses of the free substance and of the hydrochloride point to a content of 7; the data for the hydrobromide, however, agree better with 6.

Analytical data:

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[6] Watson, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 534.

[7] Heilmeyer, L., and Krebs, W., *Z. Physiol. Chem.*, 1934, **228**, 33.

1. Free stercobilin: 3.702 mg. gave 8.84 mg. $CO_2$, 2.5 mg. $H_2O$
3.207 ” ” 0.27 cc. N at 26°, 740 mm.

| | C | H | N | O |
|---|---|---|---|---|
| Theory ($C_{33}H_{46}N_4O_6$) | 66.6 | 7.74 | 9.43 | 16.16 |
| ($C_{33}H_{44}N_4O_7$) | 64.91 | 7.54 | 9.18 | 18.36 |
| Found | 65.1 | 7.58 | 9.36 | 17.96 |

2. Stercobilin hydrochloride: 3.826 mg. gave 8.60 mg. $CO_2$, 2.55 mg. $H_2O$
2.794 ” ” 0.214 cc. $N_2$ at 28°, 749 mm.
1.967 ” ” 0.43 mg. AgCl
3.064 ” ” 0.695 ” AgCl

| | C | H | N | Cl |
|---|---|---|---|---|
| Theory ($C_{33}H_{46}N_4O_6HCl$) | 62.85 | 7.46 | 8.88 | 5.55 |
| ($C_{33}H_{46}N_4O_7HCl$) | 61.3 | 7.27 | 8.66 | 5.41 |
| Found | 61.3 | 7.45 | 8.55 | 5.41<br>5.61 |

3. Stercobilin hydrobromide: 3.950 mg. gave 8.42 mg. $CO_2$, 2.48 mg. $H_2O$
2.545 ” ” 0.199 cc. $N_2$ at 31.5°, 744 mm.
3.120 ” ” 0.89 mg. Agbr

| | C | H | N | Br |
|---|---|---|---|---|
| Theory ($C_{33}H_{46}N_4O_6HBr$) | 58.65 | 6.96 | 8.29 | 11.85 |
| ($C_{33}H_{46}N_4O_7HBr$) | 57.3 | 6.7 | 8.1 | 11.57 |
| Found | 58.1 | 7.02 | 8.57 | 12.14 |

Urobilin hydrobromide (prepared in the same way from crystalline urine urobilin):
3.068 mg. gave 0.240 cc. $N_2$ at 31°, 744 mm. = 8.59% N.

A detailed report of this study appears elsewhere.[8]

## 8155 P

## Comparison of Natural Crystalline Urobilin (Stercobilin) with that Obtained in Vitro from Mesobilirubinogen.*

C. J. WATSON. (Introduced by H. A. Reimann.)

*From the Department of Medicine, University of Minnesota Hospital.*

Fischer[1] isolated mesobilirubinogen following an amalgam reduction of bilirubin. Soon afterwards he and Meyer-Betz[2] obtained crystalline urobilinogen from the urine and proved its identity with mesobilirubinogen. There is little doubt that this is the chromogen of natural urobilin or stercobilin, which recent studies[3, 4] have proven identical. It was therefore expected, after allowing crystalline mesobilirubinogen to stand in the air and light until the Ehrlich reaction disappeared and urobilin characteristics had become intense, that a crystalline urobilin could be isolated which

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[8] Watson, C. J., *Z. Physiol. Chem.*, 1935, **233**, 39.

* Aided by a grant from the University of Minnesota Graduate School.

[1] Fischer, H., *Z. Physiol. Chem.*, 1911, **73**, 204.

[2] Fischer, H., and Meyer-Betz, *Z. Physiol. Chem.*, 1911, **75**, 232.

[3] Watson, C. J., *Z. Physiol. Chem.*, 1933, **221**, 145.

[4] Watson, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 534.

would be identical with that already obtained from urine and feces. However, it appears that the natural formation of urobilin from urobilinogen is more complex than had been believed. Crystalline mesobilirubinogen (urobilinogen) was isolated from bilirubin according to H. Fischer's method.[1] It was dissolved in glacial acetic acid and remained exposed to the ordinary light and temperature of the laboratory for 3 weeks. After this time the Ehrlich reaction was only very faint. The solution now possessed a dark brown color, exhibited intense urobilin absorption and strong green fluorescence with alcoholic zinc acetate. This solution was poured into 3 volumes of chloroform, and from now on the procedure leading to isolation of the crystalline substance was the same as previously described for isolation of urobilin from urine, and stercobilin from feces.[3, 5] ($CHCl_3 \rightarrow H_2O \rightarrow HCl \rightarrow CHCl_3 \rightarrow$ petroleum ether, ppt. $\rightarrow$ hot $CHCl_3$, crystallization.) In this way a crystalline urobilin has been isolated twice in small amounts. After repeated recrystallization from hot chloroform the melting point of the crystals was not sharp at 155-158°C. Although this substance exhibits intense green fluorescence in alcoholic zinc acetate solution, as well as a "urobilin" spectrum it differs in a number of respects from natural urobilin or stercobilin. The crystals of the hydrochloride have a different appearance in that they are short and thick rectangles, often somewhat barrel shaped, in contrast to the long narrow prisms of the natural material. The substance is obviously much less stabile than the natural urobilin, and this undoubtedly accounts for the poor yields so far obtained (7-12%). An attempt was made to prepare a crystalline iron chloride molecular compound, as well as a hydrobromide, for purposes of comparison, but in both instances the substance underwent definite changes in color, in the first becoming greenish brown, in the second light red. Control experiments with smaller amounts of natural urobilin hydrochloride readily yielded the characteristic crystals of the iron chloride molecular compound and of the hydrobromide.

As yet the amount of "artificial" urobilin isolated has not been enough for microanalysis. This may yield further information as to how the natural and "artificial" urobilins differ. The differences described suggest that some as yet unknown factor is of importance in the natural transition of mesobilirubinogen to urobilin.

A detailed report of this study appears elsewhere.[6]

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[5] Watson, C. J., *J. Biol. Chem.*, 1934, **105**, 469.

[6] Watson, C. J., *Z. Physiol. Chem.*, 1935, **233**, 39.

# Western New York Section

*Syracuse University, May 4, 1935.*

### 8156 P

## Localization of Premature Beats in the Mammalian Ventricle.*

JANE SANDS ROBB, ROBERT CUMMING ROBB, AND J. G. FRED HISS.

*From the Departments of Pharmacology and Medicine, Syracuse University.*

Premature beats, excited by 2 methods, have been recorded by means of the 3 standard electrocardiographic leads, photographed simultaneously. (1) When coronary arteries are ligated, as previously reported,[1, 2] immediately upon ligation there often occur transient showers of spontaneous premature beats. Comparing the records of various experiments, it is evident that the form of the premature beats varies but that in experiments involving any one muscle the form is relatively constant. (2) In other experiments premature beats were elicited from the apex of the left ventricle by mechanical stimulation.

In the lower third of the left ventricle there are 2, and only 2 muscles. The superficial sino-spiral forms the anterior horn of the apex and penetrating becomes the anterior papillary muscle. The superficial bulbo-spiral, in a similar manner becomes the posterior papillary. If 2 types only of premature beats are obtained in this area, an anatomical basis for that fact is available.

The form of the premature beats elicited by mechanical stimulation of the superficial sino-spiral muscle was found to be the same as that of the spontaneous premature beats which occurred when that muscle lost its blood supply. Likewise the same relation between the spontaneous and artificially excited premature beats was observed for the superficial bulbo-spiral. Figure 1 shows that the

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* We gratefully acknowledge a grant from the Hendricks Research Fund of Syracuse University, a grant from the Committee on Scientific Research of the American Medical Association, and a grant from the Ella Sachs Plotz Foundation.

[1] Robb, J. S., Easby, M., and Hiss, J. G. F., *A. J. M. S.*, 1934, **188**, 835.

[2] Robb, J. S., Hiss, J. G. F., and Robb, R. C., *Am. Heart*, 1935, **10**, 287.

form of such premature beats tends to be the reciprocal of the alteration produced when respective muscles are injured.[3]

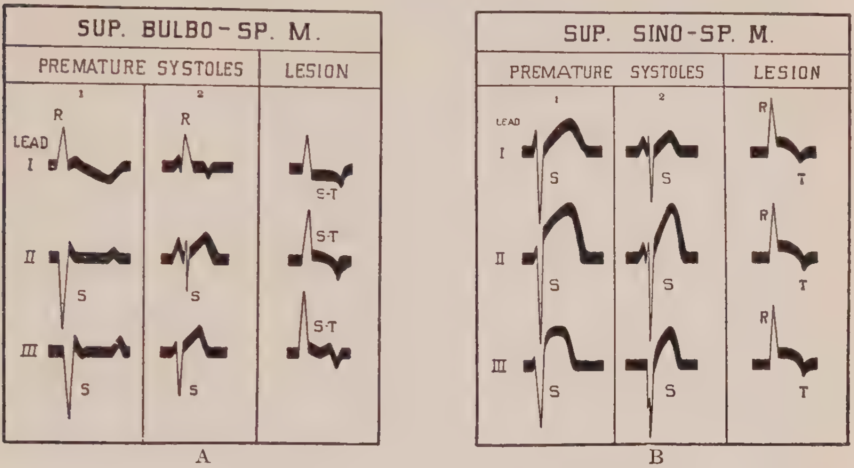

FIG. 1.

A. Superficial Bulbo-Spiral muscle. Column 1, three simultaneous leads showing mechanically stimulated premature beats. Column 2, premature beats occurring spontaneously. The column marked "Lesion" indicates the alteration due to infarction of the muscle.

B. Gives the same data for the Superficial Sino-Spiral muscle. Note that the T deflection of the premature beats is positive and that in infarction the T is negative. Also note the reverse direction of the quick deflections.

That 2 types of premature beats may be elicited from nearby points at the apex has been observed previously. Particular reference is made to figures 7a and 7b of the paper by Barker, Macleod and Alexander.[4] Their points 2 and 3 obviously lie on the superficial sino-spiral and with equal certainty points 4, 5, 6 and 8 lie on the superficial bulbo-spiral, 4 at the apex and 8 at the base. The forms of the premature beats recorded from these 2 groups of points are the same as those hereby reported. These authors discuss the previous attempts at localization of premature beats, that is, right or left sided as opposed to apex or base, and give a fairly complete bibliography.

These data allow one to suggest that localization may be possible in terms of the muscle involved.

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[3] Robb, J. S., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 311.

[4] Barker, P. S., Macleod, A. G., Alexander, J., *Am. Heart*, 1930, **5**, 720.

### 8157 P

## Transformation of Testis to Ovary in Heteroplastic Parabiosis in Amblystoma.

ROBERT K. BURNS, JR.

*From the Department of Anatomy, The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*

A study of sex reversal in heteroplastic combinations of Amblystoma has been in progress for some time, and a total of 146 pairs of *A. tigrinum—A. punctatum* have been studied, in which both partners are sexually differentiated. The advantage of heteroplastic combination lies in the more rapid development and larger size of the tigrinum member, which invariably becomes sexually dominant at a relatively early stage, regardless of its own sex. Likewise, the gonads of the punctatum member, irrespective of sex, undergo modification and reversal in varying degree. In the present paper we shall confine ourselves to an outline of the process of transformation from testis to ovary in the gonads of punctatum males joined to large tigrinum females.

In accordance with the laws of chance combination, 4 sex-species combinations are possible, and up to about 90 days in the experiment these occur in the expected ratio, as follows: ♂♂ 20, ♂♀ 17, ♀♂ 21, ♀♀ 23.* From this it is apparent that such factors as early mortality, or atypical sex ratios in the eggs used, can be excluded as influencing the result. And since the mortality subsequent to sex differentiation was low, and moreover included in the data, this factor, difficult to rule out in past experiments, is here completely eliminated from the result.

In the later stages of the experiment the expected ratio is greatly modified due to complete reversal of sex in the punctatum member of many pairs of the ♀♂ group. This is accompanied by a corresponding excess of ♀♀ pairs: ♂♂ 20, ♂♀ 13, ♀♂ 3, ♀♀ 29. These data, coupled with the histological evidence to be summarized, force us to conclude that virtually all punctatum males united to large tigrinum females undergo reversal of sex, which is, in many instances, already complete. The histological evidence may be summed up in the following principal points:

(1) Histological transformation of testis to ovary is seen in the gonads of almost all punctatum members of ♀♂ pairs prior to

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* In all cases in this paper the large sex symbol represents the tigrinum member, the smaller the punctatum.

90 days, beyond which few such pairs can be found. The only exceptions are found in a few quite young pairs.

(2) Transformation of testis to ovary may occur by "direct differentiation," in which indifferent gonads, zygotically testes, differentiate as ovaries in large parts of their extent, with subsequent reduction of the testicular portions; or, by delayed reversal in well developed testes, in which the rudimentary cortical component develops rapidly, while the medulla is removed by retrogressive changes involving cavity formation in the rete apparatus. All older ♀♂ pairs are conspicuous examples of this latter type.

(3) The occurrence in the ♀♀ group, of cases in which the rudimentary or atypical structure of the punctatum "ovary" is best explained by regarding them as completely transformed testes; an assumption required by the statistical evidence.

The demonstration of reversal of testis to ovary in this material agrees in histological details with cases described by Humphrey,[1] following orthotopic implantation of the gonad preprimordium, and Burns,[2] in homoplastic pairs of *A. tigrinum;* which have been discredited by Witschi.[3] It also parallels in its earlier aspects, an extensive new series by Humphrey.[4] In a recent article Witschi[5] refers to some *A. tigrinum—A. maculatum* (punctatum) pairs, as yet undescribed, in which in ♀♂ pairs "the maculatum male comes partly under the dominance of the tigrinum female . . . . to the extent that an ovarial cortex is permitted to develop . . ." This statement is entirely inadequate to describe the degree of transformation found by us, possibly because that writer has thus far encountered only the earlier stages of the process.

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[1] Humphrey, R. R., *J. Exp. Zool.*, 1929, **53**, 171.

[2] Burns, R. K., Jr., *J. Exp. Zool.*, 1931, **60**, 339.

[3] Witschi, E., *J. Exp. Zool.*, 1933, **65**, 215.

[4] Humphrey, R. R., *Anat. Rec.*, 1935, in press.

[5] Witschi, E., *Biol. Rev.*, 1934, **9**, 460.

# New York Section

## 8158 C

### Effect of Walker Rat Sarcoma on Growth of Transplanted Cancer.

G. VAN DER SCHUEREN.* (Introduced by J. B. Murphy.)

*From the Rockefeller Institute for Medical Research, New York City.*

A factor has been demonstrated in extracts of certain chicken tumors which if concentrated is capable of neutralizing the filtrable transmitting agents of chicken tumors and of retarding the growth of mammalian sarcomas.[1] A factor has also been obtained from certain active normal tissues, placenta, embryo skin,[2] and testicle[3] which has a definite inhibiting action on the growth of mammalian carcinoma. The present investigation was undertaken to determine whether an inhibiting factor could be demonstrated in mammalian tumors.

Preliminary tests made with several of the transplantable tumors indicated that the Walker tumor gave the best intimation of the presence of an inhibiting factor, so the experiments were confined to this tumor. It grows in nearly 100% of inoculated rats, seldom retrogresses, and causes death of the animal in from 6 to 7 weeks Tumors of this type from 3 to 4 weeks old were finely minced, spread in a thin layer and placed in a freezing box until desiccation was complete. After being finely powdered, 1 gm. was thoroughly extracted with 10 cc. of Ringer's solution and centrifuged. The supernatant fluid was used for the tests.

The effect of this material was tested on Mouse Sarcoma 180, Carcinoma 48, and the Brown-Pearce rabbit epithelioma.

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* Fellow of the C.R.B. Educational Foundation.

[1] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1932, **56**, 107; *Ibid.*, 1932, **56**, 483; *Science*, 1932, **75**, 540.

[2] Murphy, Jas. B., and Sturm, E., *Science*, 1933, **77**, 631; *J. Exp. Med.*, 1934, **60**, 293.

[3] Duran-Reynals, F., *J. Exp. Med.*, 1931, **54**, 493; Tanzer, R. C., *J. Exp. Med.*, 1932, **55**, 455.

*Experiments with Mouse Sarcoma* 180. In the first group of experiments the tumor material was prepared by first mincing, and then after the addition of a few drops of saline solution it was squeezed through bolting cloth. This gives a finely divided cell suspension. A portion of this was mixed with an equal volume of the Walker tumor extract and allowed to stand at room temperature for 60 minutes. As control the same amount of tumor cell suspension was diluted with an equal volume of Ringer's solution and kept under the same conditions as the test material. At the end of the period, mice were injected into one groin with 0.1 cc. of the test material and in the other with an equal amount of the control suspension. The results of 10 such experiments are given in Table I.

TABLE I.

| Exp. No. | No. of mice | Tumors<br>Experiment, % | Control, % |
|---|---|---|---|
| 1 | 6 | 0 | 33.3 |
| 2 | 11 | 18.2 | 81.8 |
| 3 | 9 | 0 | 44.4 |
| 4 | 7 | 0 | 42.8 |
| 5 | 8 | 12.5 | 62.5 |
| 6 | 9 | 0 | 44.4 |
| 7 | 10 | 70.0 | 70.0 |
| 25 | 6 | 0 | 66.6 |
| 31 | 11 | 0 | 81.8 |
| 27 | 8 | 0 | 50.0 |
| Total | 79 | 12.7 | 64.5 |

It is evident from Table I that the extract yields a factor or substance which prevents or retards the growth of Sarcoma 180.

In a second group of experiments with 59 mice the tumor suspension was prepared by passage through a wire mesh, which gave a suspension composed of fairly large cell clumps. This suspension was tested in the same manner as in the first group. No evidence of inhibition was observed.

*Experiments on Mouse Carcinoma* 48. Exactly the same procedure was next applied to a dilute cell suspension of Carcinoma 48. Results may be summarized as follows: In 19 mice injected tumors grew in 36.8% in the side injected with mixtures of cell suspension plus tumor extract, while they grew in 68.4% in the control side. The experiments were then repeated inoculating grafts which had been immersed for 1 hour in the tumor extract, instead of cell suspensions. No inhibition could be observed under such conditions.

*Brown-Pearce epithelioma of the rabbit.* The tumor material was prepared in the same manner as that described above for the first group of experiments. The rabbits were shaved in both flanks, and one side was injected intradermally in several areas with the tumor cell suspension plus Walker tumor extract, and the other side with the cell suspension diluted with salt solution. The results are given in Table II.

TABLE II.

| Rabbit No. | Experiment No. of inoculations | Experiment No. of tumors | Experiment % tumors | Control No. of inoculations | Control No. of tumors | Control % tumors |
|---|---|---|---|---|---|---|
| 519 | 4 | 0 | 0 | 4 | 2 | 50 |
| 520 | 4 | 0 | 0 | 4 | 4 | 100 |
| 570 | 3 | 0 | 0 | 4 | 4 | 100 |
| 571 | 4 | 0 | 0 | 5 | 4 | 80 |
| 578 | 7 | 0 | 0 | 4 | 4 | 100 |
| 579 | 8 | 0 | 0 | 4 | 3 | 75 |
| Total | 30 | 0 | 0 | 25 | 21 | 84 |

It is evident from Table II that the extract of the Walker tumor had a marked inhibiting effect on the growth of the epithelioma.

The question arose whether the action of the extract is on the tumor cells or on the host. The problem may be a complex one, but that at least part of the inhibiting effect is due to a direct action on the tumor cells is suggested by the following experiment.

A bolting cloth cell suspension of Mouse Tumor 180 was divided into 3 equal parts. Two of them were allowed to remain in contact with the Walker tumor extract for 10 and 75 minutes respectively before inoculation. The 3rd portion was allowed to stand for 75 minutes and was mixed with the extract 10 minutes before injection. These tests were controlled by injections of similar cell suspensions diluted with saline solution. These were injected into the opposite side of the same mice which had received the test inoculations. Results are given in Table III.

TABLE III.

| Material injected | No. of mice | Tumors Experiment % | Tumors Control % |
|---|---|---|---|
| Tumor cells after contact with the extract for 75 minutes | 10 | 0 | 81.8 |
| Tumor cells after contact with the extract for 10 minutes | 13 | 84.6 | 84.6 |
| Tumor cells after standing 75 minutes and mixed 10 minutes with the extract | 10 | 60.0 | 40.0 |

Table III shows a marked inhibiting effect when the cells had remained in contact for a long time with the extract, while no evidence of inhibition was found when the duration of contact was short. On the other hand, treatment of mice bearing either the Sarcoma 180 or Carcinoma 48 with the Walker tumor extract injected intraperitoneally had no effect. A possible inhibiting action on cells of the 180 tumor of desiccated extracts of the Brown-Pearce epithelioma of the rabbit. Sarcoma 180, Carcinoma 48, and a mouse melanoma has also been investigated. Results were consistently negative.

*Conclusions.* An extract of desiccated Walker rat tumor under certain experimental conditions prevents or retards the growth of Mouse Sarcoma 180, Mouse Carcinoma 48, and the Brown-Pearce epithelioma of the rabbit implanted in the skin. The action seems to be at least partially due to a direct effect on the cells, and is not shared by extracts of other transplantable tumors studied.

## 8159 C

## Reaction of Spontaneous Mouse Carcinomas to Blood-Carried Bacterial Toxins.

F. DURAN-REYNALS. (Introduced by J. B. Murphy.)

*From Rockefeller Institute for Medical Research, New York City.*

We reported[1] a study on the vascular reactions of a series of growths ranging from benign embryomas and granulomas to very malignant transplantable carcinomas and sarcomas of mice and rats to a blood-carried *B. coli* toxin of low potency. The phenomenon of the tumor reactivity to bacterial toxin was first described by Gratia and Linz,[2] and confirmed by Shwartzman and Michalowsky.[3] The reaction shows the general characteristics of the Shwartzman phenomenon. Apitz[4] has further proved that still other factors capable of inducing the latter phenomenon in rabbits also induce the phenomenon of the tumor reactivity.

From our investigations we concluded that only those growths showing at the same time *malignancy* and *rapidity of growth* present the phenomenon of Gratia and Linz.

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[1] Duran-Reynals, F., Proc. Soc. Exp. Biol. and Med., 1933, **31**, 341.

[2] Gratia, A., and Linz, R., *Compt. rend. Soc. Biol.*, 1931, **108**, 427; *Ann. Inst. Pasteur*, 1932, **49**, 131.

[3] Shwartzman, G., and Michalowsky, N., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 737.

[4] Apitz, K., *Z. Krebsforsch.*, 1933, **40**, 50.

As a corollary to this principle it was found on further analysis that the degree of pre-existing necrosis is, generally speaking, a good index of the degree of tumor susceptibility. Necrosis is the expression of a special state of vascular fragility which renders the rapidly growing tumor vulnerable to the injected toxin, even if the latter is practically harmless for the normal animal. Apitz[3] had already noticed a definite relation existing between the microscopic areas of necrosis and the incidence of hemorrhages.

The tumors most susceptible to the *B. coli* toxin were the fast-growing malignant transplantable tumors of rats and mice. As a result of the phenomenon a good many tumors regressed partially or totally. Regression was also observed after an apparently negative reaction in the tumor. On the other hand, the slower growing malignant spontaneous carcinomas of mice were found practically non-susceptible and their further course was not modified at all.

It is obvious that the phenomenon is conditioned by 2 sets of factors: the intrinsic factor depending on the sensitivity of the tumor itself, and the extrinsic one, depending on the activity, quantity, and route of inoculation of the toxin. Additional studies showed that both sets of factors could vicariously substitute for each other to a rather large extent. The very susceptible tumors required smaller doses of toxin to produce the same effect, and the same result was also obtained with filtrates of low toxicity as with much more active filtrates. Also, the route of inoculation did not seem to be a matter of much importance. This suggested that perhaps one could obtain with the refractory spontaneous tumors results similar to those obtained in transplantable tumors, but by using filtrates of higher toxicity.

Accordingly a series of tests was carried out on 52 mice bearing mammary carcinomas using a very toxic "agar washing" of human typhoid bacillus (supplied by Dr. Shwartzman), and filtrates of 6-day-old broth cultures of Mouse Typhoid I (*B. enteritidis*), Mouse Typhoid II (probably *B. paratyphosus* B.), and Mouse Typhoid III. The filtrates of Strain II of mouse typhoid were also very toxic; the filtrates of the other two strains were less so. The malignant character of the tumors was histologically confirmed in every case, either from biopsy or at the post-mortem examination.

In the first experiment 3 mice were injected intraperitoneally with 0.5 cc. of filtrate of the "agar washing" of the human typhoid. The 3 animals died in less than 6 hours without any alteration in their tumors.

In the second experiment 13 mice were first injected with 0.1 cc.

TABLE I.

Effect of Injection of Toxin of Mouse Typhoid Bacillus on Spontaneous Carcinomas of the Mouse.

| | No. of mice | Size of tumors | | Intensity of phenomenon in tumor after 1st injection | | | Dead within 48 hours | Evolution of tumor in survivors | | | Partial or total inhibition |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Large* | Small† | Strong | Mild or doubtful | Negative | | Steady growth | Partial inhibition | Total regression | |
| | | | | | | | | | | | % |
| 1st series<br>1st injection .25-.5 cc. intraperiton. followed by 3-9 similar weekly injections .1-.2 cc. | 22 | 16<br>(72%) | 6<br>(27%) | 11<br>(50%) | 5<br>(22%) | 6<br>(27%) | 11<br>(50%) | 5 | 4 | 2 | 27 |
| 2nd series<br>1st injections of 0.1 cc. intraperiton. or sub. cutan. followed by 3-9 similar weekly injections | 14 | 2<br>(14%) | 12<br>(85%) | 4<br>(28%) | 2<br>(14%) | 8<br>(57%) | 1‡<br>(7%) | 5 | 4 | 4 | 57 |
| Total | 36 | | | | | | | | | | 38 |

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* Tumors of a size from 2.5 x 3.0 cm. to 3.5 x 4.0 cm.

† Tumors of a size from 0.4 x 0.6 cm. to 1.5 x 2.0 cm. The few tumors of an intermediate size have been classed with one group or another according to their special growth characteristics.

‡ Mouse already sick before injection.

of toxin from Mouse Typhoid II intraperitoneally, and this was repeated at weekly intervals. The following results were obtained. Nine mice died within the first 6 days, 4 of which showed hemorrhages in their tumors. Of the 4 remaining 2 showed a weak phenomenon and the growth of the tumors was not modified. One mouse showed no reaction, but there was a temporary retrogression of the tumor. The last mouse had a medium sized papillary adenocarcinoma which retrogressed to a small nodule, proving on histological examination to be made up only of connective tissue.

In the third and most extensive experiment 36 mice were treated with a mixture of equal parts of toxin and of the 3 strains of mouse typhoid. Mice have been classified in 2 groups according to the amount of toxin given in the first injection. Simply by chance it so happened that the animals treated with the higher doses were also those bearing larger tumors, so that these 2 factors must be considered together. The details and results of the test are also given in Table I.

The group of mice having larger tumors and receiving the larger dose of toxin had a higher incidence, greater intensity of local reaction, a higher early mortality, and a lower percent of partial, or total inhibition than did the group with the preponderance of small tumors, receiving the smaller doses of toxin.

These observations duplicate similar findings on transplantable rapidly growing malignant tumors.

Moreover, Table I shows that inhibition is better observed when small or medium sized tumors are treated with small doses of toxin. The mortality under such conditions is very low. It is noteworthy that inhibition is observed without any apparent reaction taking place in the tumors and, consequently without any danger to the life of the animal. Thus, out of 14 cases of partial or total inhibition 3 gave a strong tumor reaction, 4 gave mild or doubtful reactions, and 7 negative reactions. The average length of life from the beginning of treatment in mice showing partial inhibition was 80 days, while the average for mice whose tumors were not inhibited was 50 days. The average length of life in the 6 mice showing total inhibition was 171 days. In 3 of them no tumor could be found at autopsy. In the other 3 cases the tumor recurred after the mice had remained free from disease for 46, 60, and 180 days respectively.

The facts brought to light in transplanted as well as in spontaneous tumors establish the principle that the newly formed vessels of malignant growth, either as a consequence of an excessive permeability or through some other cause, are extremely sensitive to injections of blood-carried bacterial toxins and it is this fact which creates a very special state, the *tumor vulnerability* which is responsible for the regression of malignant growths.

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### 8160 C

## Inhibiting Action of Placenta Extract on a Transplantable Malignant Epithelioma of the Rabbit.

G. VAN DER SCHUEREN.* (Introduced by J. B. Murphy.)

*From the Rockefeller Institute for Medical Research, New York City.*

Previous work has been reported showing the existence in mouse placenta of a factor markedly inhibiting the growth of both transplantable and spontaneous carcinoma of the mouse.[1] Placenta from rats and rabbits were later found to be equally effective in retarding the growth of transplantable mouse carcinoma.[2] The present study is an extension of this observation to include a tumor of a different species.

The epithelioma of Brown-Pearce was selected for this work. The tumor is extremely malignant and invasive when grafted into the testicle but if inoculated into the skin it eventually regresses after a period of active growth.

The mouse placenta was prepared according to the technique described in the above-mentioned publications. Late term placenta was finely minced, dried *in vacuo* and kept in the cold room. The desiccated material was ground, extracted with 10 volumes of Ringer's solution or plain water and then centrifuged; the supernatant fluid was used as such or diluted with 10 volumes of Ringer's solution. In some cases the extract was heated to 48°C., a procedure which did not seem to modify in either direction the activity of the extract.

The tumor cells from healthy testicle growths were passed through a masher and suspended in a few cc. of Ringer's solution. This suspension was squeezed through bolting cloth and divided into 2 parts. These were mixed with an equal volume of placenta extract and Ringer's solution respectively. The length of contact varied from 10 to 60 minutes at room temperature.

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*Fellow of the C.R.B. Educational Foundation.

[1] Murphy, Jas. B., and Sturm, E., *Science,* 1933, **77**, 631.

[2] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.,* 1934, **60**, 293.

In the first group of tests the mixtures were injected intradermally into each side of the depilated flank of the same rabbit. No more than 6 mixtures were tested in each side. The results are shown in Table I.

TABLE I.

| Substance injected with tumor cells | No. of injections | Tumors % |
|---|---|---|
| Ringer's solution | 27 | 81.5 |
| Undiluted placenta extract | 20 | 25.0 |
| Placenta extract at 1:10 | 14 | 42.8 |

It is evident from Table I that undiluted placenta extract has a marked effect in reducing the incidence of successful inoculations of the epithelial tumor. This action is partially lost after the extract has been diluted 10 times.

In a second group of tests the mixtures were injected into the testes instead of the skin. Ten rabbits were thus injected, 6 with the mixtures containing placental extract and 4 with the Ringer's solution mixtures. All of the animals developed tumors in both testicles and also metastases, without any significant difference between the two groups.

The above-described test was repeated with another placental extract, using 2 rabbits for the test injections and 4 for the control injections. None of the animals showed any apparent local growth, but autopsies after 30 days showed that the 4 control rabbits all had intensive metastases while the 2 test animals were entirely free from any evidence of disease. The number of animals here is too small to be of value except as a possible indication for further work.

*Conclusions.* Extracts of desiccated placenta contain a factor which markedly reduces the incidence of successful inoculations of an epithelial transplantable tumor in the rabbit growing in the skin, where under ordinary conditions the tumor eventually regresses. There are no indications of such strong action on the tumors growing in the testicle, in which organ the growth shows an extremely malignant character.

### 8161 P

## Enhancement of Pathogenicity of Human Typhoid Organisms by Mucin.

GEOFFREY RAKE.

*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*

It is recognized that experimental work on typhoid fever has been handicapped by the lack of a small susceptible laboratory animal. Even with highly virulent strains of *B. typhosus,* several million organisms are required to kill mice when inoculated intraperitoneally. With such numbers, the fate of the animal is due not only to virulence and invasiveness but also to toxicity from products of disintegration of the organisms. Recently Norton and Dingle[1] have been able to increase the virulence of *B. typhosus* 20,000 times by intracerebral inoculation. By combining the typhoid organisms with mucin, as used for other organisms,[2-6] their virulence for mice can be increased a million times.

The method for the preparation of mucin has been given elsewhere.[4, 6] Twelve- to 15-hour cultures of the organisms on plain agar have been used throughout. Thirteen cultures of *B. typhosus* and 2 each of Paratyphoid A and B have been tested. Five *B. typhosus* cultures were isolated within 9 months and of these, 3 required only 10 organisms to kill while 2 required 100. One strain was 2 years old and required 1,000 organisms to kill; while the other 7 were old stock strains and all failed to kill with 100,000 organisms (the largest number tested) save one, the so-called Rejuvenated Rawlins strain, which required only 10 organisms.* The Paratyphoid strains were old stock cultures. Three failed to kill with 100,000 organisms, but in the fourth 100 organisms killed. This strain had been kept frozen and dried without sub-culture for years. In the case of the stock strains that failed to kill, Rough, Normal and Smooth, motile and non-motile forms were noted. All of the virulent strains were smooth and motile. Comparative tests with the same virulent strains suspended in broth and mucin showed an increase in virulence of from 100,000 to over 1,000,000 times. It will be noted that the virulence of the stock strains was not enhanced to a like degree, suggesting that a great part of their activity is due to the breaking down of the large numbers of organisms. Attempts to enhance the virulence of strains by growth in media containing mucin have not been successful.

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[1] Norton, J. F., and Dingle, J. H., *Am. J. Pub. Health,* 1935, **25**, 609.

[2] Nungester, W. J., Wolf, A. A., and Jourdonais, L. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 120.

[3] Miller, C. P., *Science,* 1933, **78**, 340.

[4] Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1136.

[5] Rake, G., *J. Exp. Med.,* 1935, **61**, 545.

[6] Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1175.

* This strain and others were obtained from the Army Medical Center at Washington through the kindness of Colonel J. F. Siler.

Protection tests have been carried out using human sera. These results have been compared with the Widal reaction of the same sera. In the protection test the serum has been inoculated intraperitoneally into mice 30 minutes before the intraperitoneal inoculation of organisms. Each mouse receives 0.5 cc. of a 2:5 dilution of serum in saline. For the Widal reaction, O and H antigens have been prepared from the freshly isolated strain of *B. typhosus* which has been used throughout for the protection tests.

Twenty-six sera have been tested. Of these, 5 came from individuals who had received typhoid vaccine from 2 to 12 years before. All sera protected against at least 10,000 killing doses (the maximum tested) and none of the mice showed any sign of sickness. The other 21 sera, from unvaccinated persons with no history of typhoid fever, can be divided into 2 groups: the first, children up to 15 years; and the second, adults above 20 years. Of the 9 children, 8 showed little or no protection while one protected against 1,000 killing doses. Of the 12 adults, 3 showed little protection, 3 protected against 100, and 6 against 1,000 killing doses. It should be pointed out, however, that the serum of unvaccinated individuals, even when it protected against death, did not prevent the mice from becoming sick save in the highest dilutions.

The Widal reaction of the serum did not run parallel with the protective power of the serum. It was never positive except with serum containing protective antibodies. On the other hand, 2 sera which protected against 1,000 killing doses failed to show any agglutination with the antigens used. It would seem, therefore, that the protection test as described is more delicate for discovering slight serological changes than is the Widal reaction.

In brief, a method for increasing the pathogenicity of typhoid cultures for mice by the use of mucin has been determined, which offers a way of selecting cultures for the preparation of vaccines and a delicate measure of antibody changes in human sera.

## 8162 P

# Coagulating Power of Bothrops Atrox Venom on Hemophiliac Blood.

S. M. PECK, M. L. CRIMMINS, AND LOWEL A. ERF. (Introduced by Louis Gross.)

*From the Laboratories of the Mount Sinai Hospital, New York City.*

Barnet and Macfarlane[1] have recently reported on the use of snake venom for local hemostasis. Their best results were obtained with venom of the Russel viper. We have been working along similar lines with the venom of the Bothrops atrox (Fer-de-Lance). This reptile is a native of Central and South America and is fairly easy to obtain and keep in captivity. Thus, unlike the Russel viper venom, a plentiful and relatively cheap supply of this venom is readily available.

Two different samples of the dried venom were obtained through the courtesy of Dr. R. P. Connor, of the United Fruit Co. The samples represented pooled venoms from "milkings" carried out at the Serpentarium, in Tela, Honduras, several years ago. In the experiments, the venom was diluted with physiologic saline.

The minimum lethal dose for pigeons (intravenous route) weighing 250-350 gm. varied from 0.2-0.25 mg.; the minimum lethal dose in rabbits (intravenous route) of approximately 2 kilos, was 0.015-0.02 mg. These figures were determined on a large series of pigeons and rabbits with both samples.

V. Brazil gives 0.01 as the m.l.d. for pigeons and 0.07 mg. as the m.l.d. for rabbits We have consistently found, as can be seen from our figures, that pigeons were much more resistant than rabbits to the toxins (neurotoxins) of the venom.

The haemorrhagine content of the Bothrops atrox venom was titrated according to the method of Witebsky, Peck, and Neter.[2] One sample failed to elicit hemorrhage in a 1% solution, while the other sample produced a moderate amount of echymosis in 3 minutes when 1 cc. of the 1% solution was added to the chicken embryo. It was, however, decidedly poorer in hemorrhage-producing substance than Moccasin venom.

The coagulating power of Bothrops atrox venom was tested on the blood of 2 hemophiliacs: L.E., age 25, whose clotting time was

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[1] Macfarlane, R. G., and Barnet, B., *Lancet*, 1934, **2**, 985.

[2] Witebsky, E., Peck, S., and Neter, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 722.

58-60 minutes and V.B., age 36, whose clotting time varied from 1 hour and 40 minutes to 1 hour and 46 minutes. The coagulation time was determined according to the method of Lee and White. To 2 cc. of hemophiliac blood, 0.5 cc. of the Bothrops atrox venom was added in dilutions up to 1:10,000,000, and the time of clot formation was noted.

The optimum concentration of the Fer-de-Lance venom, for coagulation was in the 1:10,000 dilution. Since 0.5 cc. of the venom was added to 2 cc. of blood, the final dilution was 1:50,000. It is of interest to note that the coagulation power of the venom increased with the dilution up to 1:10,000 and then diminished. It took a much longer time to clot the blood of the older hemophiliac whose clotting time was much longer than that of the younger man. The clots when formed, were firm and retracted well. In the case of L. E. it took only 15 seconds to cause the formation of a firm clot on the addition of 0.5 cc. of the 1:10,000 dilution. The venom was still active on this patient's blood in a dilution of 1:1,000,000, 1:5,000,000 decreasing the coagulation time by more than one-half.

When one compares the optimum which we obtained with the report of Barnet and Macfarlane on the Russel viper, it seems that the Fer-de-Lance venom was apparently more efficient in producing a rapid coagulation, since the coagulation time in their patient, as obtained by their method, was only 22 minutes. To test the possibility that Moccasin venom might also act as a coagulant in high dilutions, 0.5 cc. of Moccasin venom in dilutions of 1:2,000, 1:3,000, 1:5,000, 1:10,000, 1:15,000, and 1:20,000 were added to 2 cc. of the blood of L.E. (clotting time 60 minutes). 0.5 cc. of a 1:10,000 dilution of Moccasin venom kept the blood fluid past the 60-minute period, while 0.5 cc. of the 1:20,000 dilution acted like a saline control, in that it either produced no change in the clotting time or prolonged it somewhat.

In order to compare the coagulating action of Bothrops atrox venom with tissue extracts, a rabbit lung was perfused to eliminate as much blood as possible and the lung tissue was dried to a powder in the desiccator. A 1% solution of the lung powder in physiologic saline was made. The blood of V.B., a hemophiliac, used in the previous experiment, whose coagulating time, April 19th, was one hour, was used. The Bothrops atrox solution was 61 days old. As before, 0.5 cc. of a 1:10,000 and a 1:100,000 dilution of the venom was added to 2 cc. of V.B.'s blood, and to a parallel series of 2 cc. quantities of this patient's blood, was added 0.5 cc. of a 1:10,000 and a 1:100,000 lung powder solution. Clotting occurred in 1½ minutes with the 1:10,000 dilution, and in 4 minutes with the 1:100,000 dilution of venom. The 1:10,000 dilution of lung powder caused clotting in 47 minutes, while the 1:100,000 dilution showed no clotting up to the end of the experiment. To test the activity of the lung powder, 0.5 cc. of the original 1% solution of lung powder was added to 2 cc. of the hemophiliac blood, and a firm clot was formed in 6 minutes. The clot following the addition of venom was firm and contracted very rapidly after clotting began, even with the 1:100,000 dilution. The clot formed with the lung powder was soft and easily broken up, and there was no contraction. In spite of the fact that the clotting time of V.B. had decreased, it took 1½ minutes for the 0.5 cc. of the 1 :10,000 dilution to produce clotting. This was due to the fact that in the previous experiment a fresh solution of the venom was made, and in 61 days since the preparation of the 1 :10,000 venom dilution, there was deterioration of its coagulating properties.

Since the venom is intended for use as a local hemostatic and since venoms often contain bacteria, the effect of filtration through a Zeiss or a Berkefeld filter on coagulating power, was studied. In both instances, filtered solutions were sterile. While the toxicity (neurotoxicity) was reduced as tested on pigeons the coagulation power of the venom was not interfered with as far as could be determined on hemophiliac blood. This confirms similar observation by Barnet and Macfarlane with the Russel viper.

## 8163 P

## Time Curve of Insulin Action in the Depancreatized Dog.

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Scott and Dotti[1] have made an extended study of blood sugar lowering effects of insulin given subcutaneously to rabbits. They have concluded that the results, when expressed as the fraction of sugar removed, are independent of the initial blood sugar while the absolute value of blood sugar after a given time interval or the absolute value of the fall in blood sugar is dependent on the initial value.

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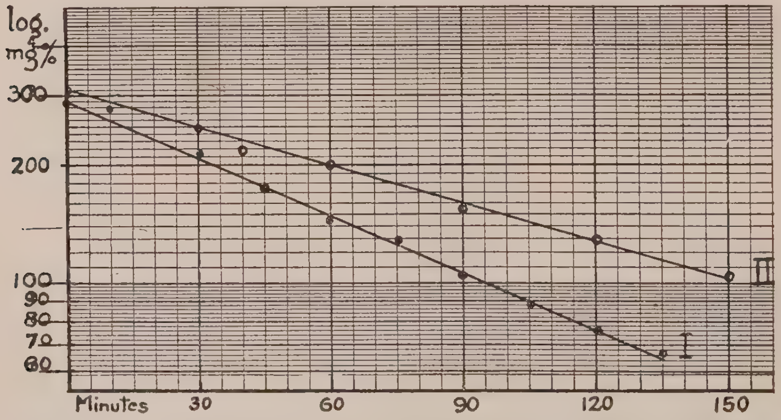
[1] Scott, E. L., and Dotti, L. B., *Arch. Int. Med.*, 1932, **50**, 511.

It is, therefore, the fraction removed and not any of the absolute values which is related only to the insulin dose. They have studied the effect after one given time interval which is empirically chosen. Observations taken at later time intervals cannot be used because the blood sugar under their conditions tends to return too early towards the initial level.

Elsewhere[2] we have stated that after pancreatectomy in the dog the blood sugar in response to intravenous insulin falls continuously for a much longer period of time than in the normal animal. The factor which tends to early return towards the initial level has been eliminated. Under favorable conditions (relatively refractory animal) and with large doses of insulin a continuous drop in sugar can be observed for more than 2 hours. If under these conditions the observations of Scott hold for the entire course of the curve, the results should show a linear relation between the logarithm of the blood sugar and the time. The graph, Curve I, shows an example of a time curve after 10 units of insulin given intravenously in a depancreatized dog. By intravenous administration we reduce materially the latent period and eliminate any effects resulting from a possible gradual absorption from the site of injection. In the diabetic dog the duration of action of insulin is markedly dependent on the dose. It is therefore only after large doses that curves having the requisite number of observations over a sufficient period of time are obtained. With smaller doses the points will also fall along a logarithmic curve but the time range of observation is limited.



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[2] Berg, B. N., Gross, J., McAfee, J., and Zucker, T. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1080.

We have so far obtained a large number of curves (over 50) with small doses, giving a blood sugar minimum at about 60 minutes. Due to the small number of points these curves are not conclusive. Twelve curves of 90 minute duration are satisfactory and 9 curves of 120 minutes or more all show good agreement with calculated values.

Curve II is plotted from data of Collens and Grayzel[3] obtained on human diabetics after 1/20 unit of insulin per kilo is given intravenously. It will be seen that this also gives quite an acceptable linear relation between the logarithm of blood sugar and time. The conclusions which the authors draw from their observations, namely that on the basis of absolute values of change in blood sugar the action of insulin in the diabetic is more marked than in the normal would be considerably modified if the principles laid down by Scott and Dotti were given consideration.

## 8164 P

## Chest Leads in Normal Children.*

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*From the Cardiovascular Laboratory, Mount Sinai Hospital, New York City.*

Chest leads in the electrocardiogram are now used for research purposes and often for the diagnosis of coronary artery thrombosis and rheumatic fever. Chest leads of normal adults have been published[1, 2] but as yet no control records of children are available.

Seventy-one normal children, from 2 to 15 years of age, 36 males and 35 females, were electrocardiographed. Seven anterior chest positions were studied and form the basis of this report. Position 1 was 2 cm. to right of midsternal line; position 2 was over the midsternum; 3, was 2 cm. to left of midsternum; 4, —4 cm. to left; 5, —6 cm. to left; 6, —8 cm.; and position 7, —10 cm. to left of midsternum.

The chest electrode was placed over the 4th intercostal space in

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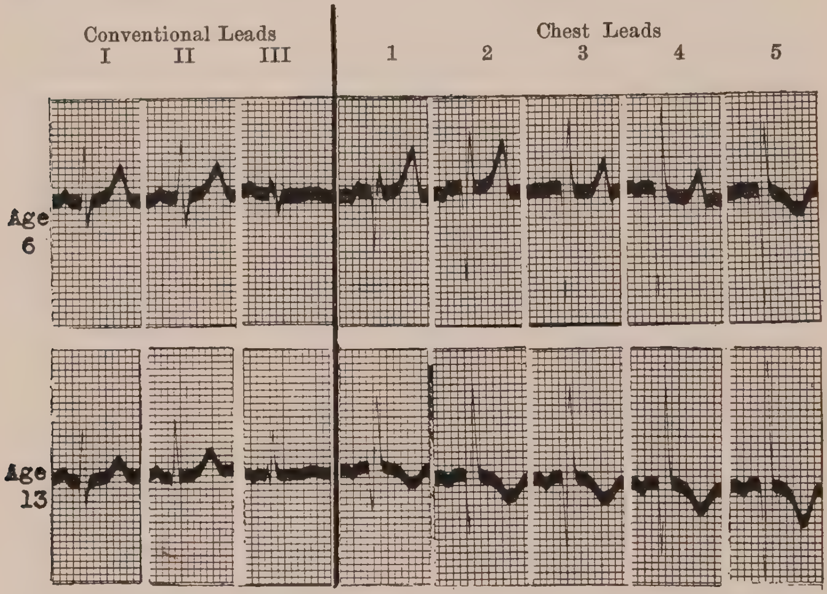
[3] Collens, W. J., and Grayzel, H. G., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 487.

*This investigation was made possible by grants from Gov. Herbert H. Lehman, Mrs. Charles and Mr. Frank Altschul.

[1] Katz, L. N., and Kissin, M., *Am. Heart J.*, 1933, **8**, 595.

[2] Master, A. M., *Am. Heart J.*, 1934, **9**, 511.

the younger children and over the 5th space in older children, corresponding to the position of the apical impulse. All children were in the reclining position. The anterior electrode[2] was a glass funnel 1 in. in diameter, in which coils of copper wire in a saline moistened pad made contact with the skin. The second or inactive electrode was applied to the left leg. This procedure[2] has been outlined before.



Position I, II, III, conventional leads.
Position 1, right of sternum; 2, over sternum; 3, left of sternum; 4, within the apex; 5, at the apex.

The "average limits" hold for 90% of the cases except for the height of the P-waves, where the averages occurred in 75%.

*P-Waves.* The height varied between —1 and +½ mm. but the wave was often absent. Negative P-waves predominated in all positions. Positive P-waves were more common at the apex than at the sternum. *The P-Q Interval.* The average duration was 0.10-0.14 second. *The Q-wave* was never absent. It increased in size from sternum toward the apex. The average amplitude was 2 to 22 mm.; the extreme limits were 1 to 33 mm.

*R-Wave.* The average limits were 2 to 22 mm. There was a tendency to decrease in size from sternum to apex, beyond which it was occasionally absent. Extremes of ½ to 40 mm. were observed. *Notching and slurring* of the QRS-waves were not abnormal and may occur in any position. *An initial upward deflection* of the QRS occurred only at or outside the apex, never over the sternum. *The QRS duration* measured 0.06-0.08 second. *R-T transition interval.* No elevation or depression over 1 mm. was found in any position. Elevation is common over the sternum but may occur at the apex; the reverse is true of depression. *T-Waves.* A positive or diphasic T-wave occurred in 30% of the cases at the apex and in 60% within the apex. This is in distinct contrast to the findings in adults in whom a positive T-wave is definitely abnormal. Upright T-waves were most frequent over the sternum, decreased progressively toward the apex and disappeared completely outside the apex. With increasing age there was a tendency for the T-wave to be inverted at the apex, *i. e.*, it became the adult type. (See Fig. 1.) The extreme limits for size were —6 and +8 mm.

*Conclusion.* Positive T-waves at, or within the apex, which are abnormal findings in adults, are normal in children.

## 8165 P

## Action of Gonadotropic Hormone from Pregnant Mare's Serum on Ovaries of Rhesus Monkeys.*

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*From the University Institute for General Pathology, Copenhagen.*

Since the demonstration of Cole and Hart[1] that the blood serum of pregnant mares contains large amounts of gonadotropic hormone, this hormone has been the subject of much investigation. Although the recent work of Catchpole and Lyons[2] indicates a placental origin of the hormone, it has been found to have biological effects similar to those found in the pituitary gland and to differ markedly from the chorionic hormone of human pregnancy. Thus the experiments of Evans, *et al.*,[3] have shown that the hormone of the pregnant mare is able to cause a marked enlargement of the ovaries of hypophysectomized rats, and it has been found to possess a pronounced stimulating effect on the testes and the comb growth of infantile cocks (Hamburger[4]) just like the anterior pituitary hormone, while the gonadotropic hormone from the urine of human pregnancy is ineffective in this respect.

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* We wish to thank August Kongsted, President of the "Løvens kemiske Fabrik," Copenhagen, not only for the hormone preparations but also for supplying the monkeys for this experiment.

† Columbia University, New York City.

[1] Cole, H. H., and Hart, G. H., *Am. J. Physiol.*, 1930, **93**, 57.

[2] Catchpole, H. R., and Lyons, W. R., *Am. J. Anat.*, 1934, **55**, 167.

[3] Evans, H. M., Meyer, K., and Simpson, M. E., *Mem. Univ. Calif.*, 1933, **11**.

The difference between the effects of extracts of human pregnancy urine and extracts of anterior pituitary or from castrate urine on the ovary of the monkey has been previously reported (Engle, Smith and Engle[5]).

It has been of interest, therefore, to investigate the effects of an active extract of the serum of pregnant mares on the follicular structure and activity of the ovary of the macacus monkey. Six females, 5 preadolescent and one adult, have been used in this preliminary experiment, the details of which are set forth in Table I.

TABLE I.
Response of Ovaries of Macacus Monkeys to Gonadotropic Hormones.

| Animal No. | Wt. gm. | Wt. left ovary, gm. | Treatment | | Sex skin change | Wt. right ovary, gm. |
|---|---|---|---|---|---|---|
| | | | Daily dosage | No. days | | |
| 1 | 3200 | .075 | 100 MU "Antex" | 10 | ± 6th day<br>+ 8th " | 1.085 |
| 2 | 3040 | .068 | 80-100 MU C. U. | 10 | ± 6th "<br>+ 8th " | 0.549 |
| 3 | 3200 | .129 | 200 MU "Antex" | 10 | + 6th day<br>Max. 9th day | 0.995 |
| 4 | 3400 | .065 | 500MU "Antex" | 10 | + 5th day<br>Max. 11th day | 0.915 |
| 5 | 4450 | .099 | 200 MU "Antex" s. c.<br>300 MU "Antex" i. v. | 5<br>5 10 | + 4-5th day<br>Max. 11th day | 1.985 |
| 6 | 2960 | .457<br>removed on day 6th | 200 MU "Antex" s. c.<br>400 MU P. U. i. v. | 5<br>5 10 | + 5th day<br>Max. 8th day<br>fading 11th day | 0.802‡ |

‡ Not yet studied microscopically; gross examination showed several hemorrhagic follicles.

"Antex"—preparation of serum of pregnant mares, "Løvens kemiske, Fabrik," Copenhagen.

C.U.—extract of urine of menopausal women.

P. U.—extract from urine of pregnant women (prepared by "Løvens kemiske Fabrik").

The results have indicated that, regarding the functional activity and structural changes of the ovary of the monkey, the active principle of pregnant mare's serum closely resembles that found in urine of castrate women. The oestral response of the sex skin of the monkey, seen on day 5 or 6 of treatment, is quite similar to that obtained by oestrin, A.P. or C.U. (Allen, Hisaw *et al.*, Engle), and progresses characteristically until the conclusion of treatment after 10 days.[6]

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[4] Hamburger, C., *Endokrinologie*, 1934, **13**, 21.

[5] Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 350; Smith, P. E., and Engle, E. T., *J. Pediatrics*, 1934, **5**, 163.

This action of a hormone produced only in an animal with living chorionic tissue, is remarkably different, on this particular test animal, from that gonadotropic substance in the human which is also found only in the presence of chorionic tissue.

Microscopically, the ovaries of the monkey, under the stated conditions, show very healthy conditions of the granulosa, as reported previously for monkeys treated with C.U. (Smith and Engle). There has been after injections of mare's serum, no thecal enlargement, no cystic degeneration nor marked atresia of ova. The only constant morphological change has been growth and proliferation of the granulosa of all medium sized and large follicles, with the immediate and consequent appearance of oestral changes in the sex skin.

Cole, *et al.*,[7] have reported, and we have confirmed, the observation, that the genital apparatus of immature male rats is enlarged after treatment with pregnant mare's serum. In our experience with 4 litters of 22-day-old male rats, each litter injected for 10 days with either mare's serum or P.U., the hypertrophy of the accessory apparatus was similar with both mare's serum and P.U. Histologically, it was impossible to determine any difference in degree of hypertrophy of the interstitial cells, or condition of the tubules of the testes of rats treated with mare's serum or with P.U.

Thus the same preparation which in the immature male rat produced a response resembling P.U., when given to immature macacus monkeys, causes an effect apparently identical with that induced by the urine of human castrates.

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[6] Meyer, R. K., and Gustus, E. L., (*Science*, 1935, **81**, 208) have also secured in the rhesus monkey great follicular development and a response of the sexual skin from injections of pregnant-mare serum extract. In their experiments there was regression of the ovarian and sexual skin response with long continued treatments.

[7] Cole, H. H., *et al.*, *Am. J. Physiol.*, 1932, **102**, 207.

## 8166 C

# Occurrence of Poliocidal Substances in Tears, Saliva and Cerebrospinal Fluid of Normal Individuals.*

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*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.*

While the presence of poliocidal substances in the serum of normal individuals is generally known and this fact is tentatively put in correlation with the mechanism of resistance to the disease, there are but few data to indicate the extent of distribution of similar substances in other body fluids and secretions of man. Apart from the early work of Amoss and Taylor,[1] who reported neutralization of poliomyelitis virus by the nasal secretions of healthy persons, and more recent observations on the inactivation of the virus by pregnancy urine, placenta extracts and various endocrine preparations,[2, 3] investigations in this field have been strangely neglected. Yet it is clear that the question whether or not virucidal substances may normally be found at the site of the presumable portal of entry or in the vicinity of the ultimate seat of the lesions, carries far greater significance than the successful demonstration of such substances in the circulating blood. In an attempt to bridge this gap in our knowledge, we have conducted a series of experiments in order to determine whether poliocidal substances are present in the tears, the saliva and the cerebrospinal fluid of normal adults.

*Virucidal tests with normal human tears.* Human tears were collected on the day of the experiment from a group of 5 or 6 adult persons after local irritation of the conjunctiva by mustard oil or onion juice. The individual specimens were combined and neutralization tests carried out with the pooled mixtures. The mixtures were used undiluted and in dilutions ranging from 1:2 to 1:20. Amounts of 0.8 cc. were combined with 0.2 cc. of a 10% suspension of poliomyelitis virus. After incubation of 1½ hours at 37°C., the tears-virus mixtures were allowed to stand in the ice-box for 24 hours and then injected intracerebrally into individual monkeys. A total of 11 tests were carried out, 2 with undiluted tears, 4 with

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* Under a grant from the Rockefeller Foundation.

[1] Amoss, H. L., and Taylor, E., *J. Exp. Med.*, 1917, **25**, 507.

[2] Jungeblut, C. W., Meyer, K., and Engle, E. T., *J. Immunol.*, 1934, **27**, 43.

[3] McKhann, C. F., and Chu, F. T., *J. Inf. Dis.*, 1933, **52**, 268; *Am. J. Dis. Child.*, 1933, **45**, 475.

tears diluted 1:2, 4 with tears diluted 1:5 and 1 with tears diluted 1:20.

Both animals receiving the undiluted tears-virus mixtures survived without paralytic symptoms. Of those injected with the tears diluted 1:2 plus virus, 2 failed to develop paralysis while 2 came down with typical poliomyelitis. In the group of the 4 monkeys injected with the tears diluted 1:5 plus virus, 2 developed paralysis after greatly prolonged incubation periods, *i. e.*, 15 and 25 days respectively, while the remaining 2 animals developed typical poliomyelitis. The last monkey injected with tears diluted 1:20 plus virus showed the usual course of the disease as seen in controls. It appears, therefore, that undiluted tears possess definite poliocidal properties, but that the virucidal effect diminishes rapidly with dilution, the threshold concentration lying in the neighborhood of a 1:2 dilution.

*Virucidal tests with normal human saliva.* Fresh saliva was obtained from the same group of persons. Individual samples were combined and the pooled saliva was filtered through a Berkefeld candle. The sterile filtrate was tested undiluted and in various dilutions for poliocidal properties as indicated above. A total of 23 tests were performed, 4 with undiluted saliva, 15 with saliva diluted 1:2, 3 with saliva diluted 1:5 and 1 with saliva diluted 1:20.

In these experiments there was only one animal surviving without paralytic symptoms, *i. e.*, a monkey which had received a mixture of saliva diluted 1:2 plus virus, although 3 additional animals developed the disease after greatly prolonged incubation periods, ranging from 15 to 21 days. Of these latter animals 1 had been injected with undiluted saliva and virus and the other 2 with saliva diluted 1:2 and virus. All other monkeys came down with typical poliomyelitis so that it can be said that in the majority of the cases there was no evidence of the presence of neutralizing substances, even when the saliva was tested undiluted or in low dilutions.

*Virucidal tests with normal human spinal fluid.* These tests were carried out with 3 pools of human spinal fluid, each containing 10 individual samples. Two of these pools consisted of Wassermann-positive specimens, while the third contained only Wassermann-negative fluids. When tested for neutralizing property according to the standard technic, none inactivated the poliomyelitis virus, although in 2 instances (Wa+ pools) the monkeys receiving the spinal fluid-virus mixtures developed only very slight transient paralysis.

*Virucidal tests with convalescent monkey spinal fluid.* In order to provide a basis of comparison with the mechanism of acquired immunity, a total of 6 specimens of convalescent monkey spinal fluid, obtained early as well as late after the onset of paralysis, were examined for poliocidal properties. The technic was the same as described above. Five specimens failed to give any evidence whatsoever of the presence of neutralizing substances. However, one monkey injected with the sixth sample, developed paralysis only after an incubation period of 28 days, suggesting a partial inactivation of the virus. This particular specimen was obtained during the acute stage of the infection.

All of these experiments were adequately controlled by the inclusion of 13 control monkeys injected with a mixture of 0.2 cc. 10% virus suspension and 0.8 cc. saline. All of these animals developed complete paralysis within from 6 to 10 days.

It would appear that, as far as our data go, the tears of normal adult persons contain regularly poliocidal substances of low titre. It seems logical to assume that the presence of such substances at the natural portal of entry signifies an important factor of local defense in protecting the nasal mucous membranes against the passage of poliomyelitis virus. The nature of these substances is obscure and we did not ascertain whether their presence coincides with the presence of poliocidal substances in the serum of the same individual. Human tears, however, are known to contain an enzymatic bactericidal principle (lysozyme) which occurs also in fresh egg white.† In human saliva, on the other hand, poliocidal substances seem to be normally absent. Occasional irregular or borderline results that were obtained in some neutralization tests may indicate the chance occurrence of minute amounts of the same substance. The possibility cannot be ruled out that their concentration might have been diminished by filtration. Normal human spinal fluid appears to be ordinarily devoid of poliocidal principles. Whether the light infections observed after injection of the Wa+ spinal fluid-virus mixtures were due to the presence of serum elements in these fluids is an attractive problem which merits further consideration. Similarly, the spinal fluid of convalescent monkeys, with rare exceptions, is evidently free from virucidal substances. In this respect our results agree with those previously reported in the literature.[4] It is rather remarkable that the spinal fluid of convalescent monkeys should lack poliocidal properties even when the serum of the same animal can be shown to possess inactivating power, as was true in these experiments. This fact is in marked contrast to the distribution of immune bodies in some other infections of the central nervous system, such as neuro-syphilis, involvement of the brain and cord, whether latent or manifest, giving rise with great regularity to the appearance of the Wassermann reagin in the blood as well as in the cerebrospinal fluid. The reasons for this discrepancy seem to be worthy of further investigation.

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† Preliminary experiments in which egg white was tested for poliocidal properties so far have given irregular results. The problem is being studied further at present and a complete report will be submitted at some later time.

[4] Poliomyelitis, Monograph of the International Committee for the Study of Infantile Paralysis, Williams & Wilkins, Baltimore, 1932.

### 8167 C

## Effect of Adrenalectomy on Poliocidal Property of Serum of Normal Cebus and Convalescent Rhesus Monkeys.*

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In preliminary experiments it was reported that the natural poliocidal property of the serum of one Nicaraguan Cebus monkey was lost after subjecting the animal to bilateral adrenalectomy.[1] It was also demonstrated that this property could be restored by the addition of a small amount of cortical hormone, but not of adrenaline, to the deficient serum. In order to lend significance to this isolated observation it became necessary to repeat these experiments on a larger scale. Furthermore, it seemed of interest to extend the scope of this work to an investigation of the effect of adrenalectomy on the virucidal property of the serum of rhesus monkeys which had recovered from a paralyzing attack of poliomyelitis.

In conducting these experiments it was soon discovered that there exists a fundamental difference between black-faced Cebus monkeys from South America and white-faced Cebus monkeys from Central America with respect to the presence of natural poliocidal substances in their serum. Thus, in keeping with previous experience,[2] the normal serum of 3 Nicaraguan Cebus monkeys produced either complete or partial neutralization of poliomyelitis virus *in vitro*, 2

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* Under a grant from the Rockefeller Foundation.

[1] Jungeblut, C. W., *J. Bact.*, 1934, **27**, 81.

[2] Jungeblut, C. W., and Engle, E. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 879.

monkeys receiving the serum-virus mixtures intracerebrally remaining entirely free from symptoms, while the 3rd animal developed partial paralysis on the 17th day. On the other hand, the normal serum of 3 Brazilian Cebus monkeys gave no evidence whatsoever of being capable of inactivating the virus *in vitro*. Three control monkeys receiving the same amount of virus, *i. e.*, 0.2 cc. 10% virus suspension, mixed with 0.8 cc. saline accompanied these tests. All developed complete paralysis within from 7-9 days.

After having demonstrated the presence of natural poliocidal substances in the serum of 3 Nicaraguan Cebus monkeys, these animals were subjected to bilateral adrenalectomy. The adrenals were exposed by a paravertebral incision, with the last rib as the upper landmark, the vessels were ligated, and both glands removed *in toto* in one stage. The immediate effects of the operation were well tolerated by all 3 animals. Symptoms of adrenal cortical deficiency set in between the sixth and ninth day, leading to death within a few hours. Three to 5 days following removal of the adrenals the animals were bled again and their serums tested for virucidal property. None of the serums obtained from the adrenalectomized animals showed any evidence of neutralizing capacity, the monkeys receiving the serum-virus mixtures developing complete paralysis, like 2 control monkeys, within from 6-9 days.

Bilateral adrenalectomies were then carried out on 3 rhesus monkeys which had retained considerable residual paralysis following a previous attack of experimental poliomyelitis. In addition, one rhesus monkey which had been actively immunized by a series of subcutaneous injections of live virus over a period of 3 weeks was subjected to the same operation. These animals were selected from a larger group of similar animals because their serums when tested before the operation possessed definite virucidal property. The 4 animals referred to above, even though the adrenals in 2 of them had been removed in 2 stages, tolerated the operation not nearly as well as the Cebus monkeys. Symptoms of cortical deficiency set in between 24 and 48 hours and caused death shortly thereafter.† Neutralization tests carried out with the serums taken after adrenalectomy indicated that in 3 out of 4 cases the serum had not lost its neutralizing capacity as the result of the adrenalectomy. The experiments described in this paragraph were adequately controlled by the inclusion of 5 control monkeys, all of which developed complete paralysis within from 6-10 days.

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† We have satisfied ourselves that the short survival time was not altogether due to the particular condition of the animals since 3 normal rhesus monkeys subjected to bilateral adrenalectomy died within from 24 hours to 5 days after the operation.

Although the number of experimental animals in the several categories is small—due to technical difficulties peculiar to this type of work—the results are nevertheless so consistent that it seems safe to draw certain conclusions. While bilateral adrenalectomy is regularly followed by a disappearance from the serum of the natural poliocidal substance, it would appear that the same operation does not ordinarily change this property of the serum in animals which have acquired it as the result of convalescence from the disease or by active immunization. It is impossible to say, however, whether this difference indicates a qualitative dissimilarity in the nature of the 2 virucidal principles involved or is caused by variations in the quantities present. At any rate, our results agree well with similar observations of Marmorston-Gottesman and Perla[3] on the effect of suprarenalecomy on the natural and acquired resistance of rats to typhoid vaccine.

## 3168 C

## A Value for the Tension at the Surface of a Myxomycete.

DARWIN VEXLER. (Introduced by E. Newton Harvey.)
*From the Laboratory of Physiology, Princeton University.*

It has appeared of interest to obtain a value for the tension at the surface of a myxomycete* in view of the fact that these organisms exhibit relatively rapid protoplasmic streaming. The method used was the oil drop one of Harvey and Marsland.[1] An oil droplet is injected into the organism and is then pulled out by centrifugal force while under observation in the microscope-centrifuge. The buoyant force of the oil is equated to the tension around the circumference of a neck of protoplasm of the same diameter as the oil drop.

$$\pi DT = V_0(\Delta\rho)CG$$

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[3] Marmorston-Gottesman, J., and Perla, D., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 648.

* The myxomycete used (Physarum polycephalum) was obtained from the University of Pennsylvania through the courtesy of Dr. W. Seifriz.

[1] Harvey, E. N., and Marsland, D. A., *J. Cell. and Comp. Physiol.*, 1932, **2**, 75.

Where $D =$ diameter of oil globule, $T =$ tension in dynes, $V_o =$ volume of oil droplet, $\Delta\rho =$ difference in density between oil and medium, $C =$ centrifugal force in times gravity, $G =$ force of gravity, 980.

The criterion of recovery was protoplasmic streaming after the experiment. It was noted that centrifuging apparently stimulates streaming. In almost all of the experiments, as the oil pulled out, a very thin pellicle was visible at the surface of the myxomycete through which the oil passed. The film coalesced again, leaving no frayed edges after the oil had separated. Once the oil starts to pull off the neck of protoplasm narrows very quickly.

The medium was frog Ringer's solution diluted 250 times. Four different oils were used, as shown in the tables. While the differences in values observed are not very great, that obtained using Menhaden oil seems significantly smaller. This may be correlated with the appearance of the oil on injection. In all cases but that of the Menhaden oil, discrete globules of oil were formed of the complete mass of oil injected. The Menhaden oil, however, formed a diffuse injection, and when centrifuged small parts of this in the form of small globules separated out and then pulled off. In control experiments, the Menhaden oil was seen to diffuse uniformly throughout the animal in 20 to 30 minutes, and was then not visible. Subsequent centrifuging resulted only in the separation of a layer of oil on the centripetal edge of the organism, not in the formation of globules which could be pulled out. It appears that both olive and paraffin oils may cause some denaturation of pro-

TABLE I.

OLIVE OIL. $T = 56.05\ Cr^2$. Density of oil, 0.914.

| Exp. | | | r (μ) | C (xg) | T (Dynes/cm.) | Recovery |
|---|---|---|---|---|---|---|
| April | 19 | 1 | 12 | 4330 | .35 | ? |
| ,, | 28 | 1 | 6 | 5870 | .1187 | + |
| ,, | 28 | 2 | 11.2 | 3950 | .278 | + |
| May | 5 | 1 | 10 | 3830 | .2145 | + |
| ,, | 5 | 2 | 16 | 3475 | .498 | + |
| ,, | 5 | 3 | 18 | 3710 | .674 | + |
| ,, | 5 | 4 | 20 | 3245 | .727 | + |
| ,, | 5 | 5 | 8 | 8350 | .299 | ? |
| ,, | 6 | 1 | 14 | 4860 | .534 | + |
| ,, | 6 | 2 | 20 | 2040 | .458 | + |
| ,, | 6 | 3 | 8 | 6170 | .221 | + |
| ,, | 8 | 1 | 44 | 386 | .418 | + |
| ,, | 8 | 2 | 35.0 | 1185 | .813 | + |
| ,, | 8 | 3 | 20 | 3589 | .804 | — |
| ,, | 10 | 1 | 20 | 3020 | .677 | + |
| ,, | 11 | 1 | 20 | 1185 | .265 | + |
| Aver. tension | | | | | .458 | |

MENHADEN OIL. T = 47.57 $Cr^2$. Density of oil, 0.927.

| Exp. | | | r ($\mu$) | C (xg) | T (Dynes/cm.) | Recovery |
|---|---|---|---|---|---|---|
| May | 13 | 1 | 12 | 1710 | .117 | + |
| ,, | ,, | 2 | 6 | 3245 | .0557 | + |
| ,, | ,, | 3 | 8 | 3475 | .106 | + |
| ,, | ,, | 4 | 12 | 1780 | .122 | + |
| ,, | ,, | 5 | 14 | 2040 | .190 | + |
| ,, | ,, | 6 | 12 | 2820 | .193 | + |
| ,, | 14 | 1 | 20 | 2040 | .389 | + |
| ,, | ,, | 2 | 12 | 2220 | .152 | + |
| ,, | ,, | 3 | 10 | 3240 | .154 | + |
| ,, | 15 | 1 | 4 | 6020 | .0458 | + |
| ,, | ,, | 2 | 6 | 6480 | .111 | + |
| ,, | ,, | 3 | 4 | 7820 | .0595 | + |
| Aver. tension | | | | | .141 | |

PARAFFINE OIL. T = 72.99 $Cr^2$. Density of oil, 0.892.

| Exp. | | | r ($\mu$) | C (xg) | T (Dynes/cm.) | Recovery |
|---|---|---|---|---|---|---|
| May | 16 | 1 | 12 | 5720 | .602 | + |
| ,, | ,, | 2 | 6 | 8350 | .2195 | ? |
| ,, | 17 | 1 | 12 | 8270 | .869 | + |
| ,, | ,, | 2 | 20 | 4580 | 1.34 | + |
| ,, | 18 | 1 | 8 | 8350 | .391 | + |
| ,, | ,, | 2 | 7 | 8350 | .299 | + |
| ,, | ,, | 3 | 8 | >8350 | >.391 | + |
| ,, | ,, | 4 | 14 | 6000 | .515 | + |
| ,, | ,, | 5 | 16 | 5360 | 1.005 | + |
| Aver. tension | | | | | 0.626 | |

50% OLIVE OIL, 50% MENHADEN OIL MIXTURE.
T = 51.81 $Cr^2$. Density of oil, 0.921.

| Exp. | | | r ($\mu$) | C (xg) | T (Dynes/cm.) | Recovery |
|---|---|---|---|---|---|---|
| May | 19 | 1 | 32 | 466 | .247 | + |
| ,, | ,, | 2 | 24 | 2222 | .664 | + |
| ,, | ,, | 3 | 22 | 3475 | .870 | + |
| ,, | ,, | 4 | 12 | 7140 | .533 | + |
| ,, | ,, | 5 | 10 | 7300 | .378 | + |
| ,, | ,, | 6 | 12 | 6970 | .520 | + |
| Aver. tension | | | | | .534 | |

tein at their surfaces. No data is available for Menhaden oil. (J. F. Danielli, private communication.)

In general the tensions found for the myxomycete are somewhat lower than those determined by Harvey and Marsland[1] for *Amoeba dubia.* The results obtained are presented in the accompanying tables.

I should like to express my deep appreciation for the interest and direction of Dr. E. N. Harvey, and also that of Dr. J. F. Danielli.

### 8169 C

## A New Nutritional Disease of the Chick Embryo.

T. C. BYERLY, HARRY W. TITUS, N. R. ELLIS, AND W. LANDAUER.

*From the Beltsville Research Center, U. S. Department of Agriculture, Beltsville, Md., and the Agricultural Experiment Station, Storrs, Conn.*

From 1930 to 1934, inclusive, a disease, not heretofore described, has caused the death of many chick embryos during the third week of incubation of eggs produced by chickens receiving certain diets and management at the Beltsville Research Center. This disease is characterized by shortness of the bones. Shortness of the antero-posterior axis of the skull and shortness of the tarso-metatarsi are particularly striking, as shown in Fig. 1. The shafts of the leg bones are extremely osteoporotic.*

Affected embryos occasionally hatch. Some of the hatched chicks behave abnormally, presenting an appearance very much like that caused by polyneuritis. A few affected chicks have been reared on normal chick diets and they lost all abnormal appearance in the course of 10 weeks.

Data on incidence of affected embryos in eggs from birds receiving various diets and management are presented in Tables 1, 2 and 3. The data presented in Table 1 are from birds of the Single-Comb Rhode Island Red variety, as are a part of those presented in Tables 2 and 3. The remainder of the data presented in Tables 2 and 3 are from $F_1$ birds from a cross between Single-Comb White Leghorns and Jersey Black Giants. The data presented are for embryos of more than 13 days' incubation and for the hatched chicks. Embryos of less than 13 days' incubation could not be classified with certainty. Eggs were incubated during each month of the year.

Each of the diets fed the birds used in the experiments summarized in Table 1 was compounded so as to contain 20% of protein and to have a Ca/P ratio of 2.5:1. Each diet also contained 0.5 part by weight of common salt, 0.5 part by weight of anhydrous sodium sulphate, and 2 parts by weight of cod-liver oil. In compounding this diet, and all diets subsequently described which contained a D supplement in oil, the dry ingredients of the diet were made up to one hundred parts by weight and the oil added in the quantities indicated.

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* A report on the histological features of the affected chicks will be presented by W. Landauer in another place.

The basal diet contained 94-95 parts by weight of a mixture of pearled hominy and meat meal. In compounding the supplemented diets, a portion of the pearled hominy and commercial meat meal was replaced by the supplement used.

TABLE I.
Incidence of short-boned embryos and chicks in eggs from birds receiving simple diets.

| Supplement | | Embryos and chicks classified | | |
|---|---|---|---|---|
| | | Total | Short-boned individuals | |
| Kind | Parts by wt. | No. | No. | % |
| None | — | 300 | 16 | 5.33 |
| Dried yeast cells | 15 | 146 | 57 | 39.04 |
| Rice bran | 15 | 1,658 | 111 | 6.63 |
| Wheat germ | 15 | 871 | 11 | 1.26 |
| ,, ,, | 15 | | | |
| Dried whey | 10 | 268 | 0 | 0.00 |
| Wheat germ | 15 | | | |
| Dried liver | 10 | 441 | 0 | 0.00 |

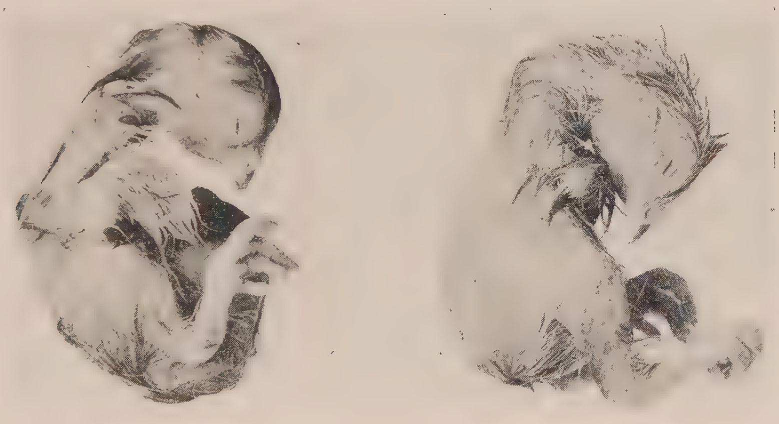

Fig. 1.
Short boned (left) and normal (right) embryos of 21 days' incubation.

The unsupplemented basal diet of Table 1 was shown by Ellis and coworkers,[1] by means of rat tests, to be very deficient in vitamins B and G. The eggs produced by the birds which received it were shown to be very deficient in vitamin B, but rat-feeding tests failed to indicate a marked G deficiency in these eggs. Barnum[2] has shown that eggs produced by birds receiving the basal diet are deficient in vitamin E. The data presented have shown that the addition of vitamins B and G by means of yeast or rice bran certainly did not reduce the incidence of short-boned embryos. On the other hand, addition of wheat germ, which is a rich source of vitamin E, greatly reduced the incidence. Supplements of whey or dried liver, in addition to wheat germ, apparently eliminated the disease.

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[1] Ellis, N. R., Miller, D., Titus, H. W., and Byerly, T. C., *J. Nutr.*, 1933, **6**, 243.

[2] Barnum, G. L., *J. Nutr.*, 1935, **9**, 621.

The diets fed the birds used in the experiments summarized in Table II contained 94-96 parts, by weight, of a basal feed mixture consisting of 50 parts, by weight, of ground yellow corn, 24.5 parts, by weight of wheat bran, 15 parts, by weight, of oat meal, and 5.5 parts, by weight, of alfalfa leaf meal. They also contained 6-4 parts, by weight, of a mineral mixture consisting of such quantities of ground limestone and steamed bone meal as were required to give a Ca/P ratio of about 2.5:1, and 0.5 parts, by weight, of common salt and anhydrous sodium sulphate, respectively. Cod-liver oil was added to the finally compounded diets in the quantities indicated in Table II.

TABLE II.
Incidence of short-boned embryos and chicks in eggs from birds which received a diet chiefly of vegetable origin with or without direct sunlight or cod-liver oil.

| Supplement | | Embryos and chicks classified | | |
|---|---|---|---|---|
| | | Total | Short-boned individuals | |
| Kind | Parts by wt. | No. | No. | % |
| Cod-liver oil | 2 | 489 | 9 | 1.84 |
| Sunlight through glass | — | | | |
| Cod-liver oil | 2 | 3,734 | 7 | 0.18 |
| Direct sunlight | — | | | |
| Cod-liver oil | 2 | 643 | 0 | 0.00 |
| Direct sunlight | — | | | |
| Range | ? | | | |
| Cod-liver oil | 0 | | | |
| Direct sunlight | — | 835 | 0 | 0.00 |
| Range | ? | | | |

The data presented in Table II indicate that direct sunlight and perhaps some substance obtained from the soil or green plants are effective in reducing the incidence of short-boned embryos. Access to range may have induced the birds to expose themselves to direct sunlight for longer periods than the birds which had access only to concrete-floored runyards.

The diets fed the birds used in the experiments summarized in Table III contained 74-76 parts, by weight, of the same basal feed mixture as that used in the diets for the experiments summarized in Table II, 6-4 parts, by weight, of a mineral mixture similar to that used in the diets for the experiments summarized in Table II which was varied to maintain a Ca/P ratio of 2.5:1, and the various supplements stated in Table III. The 3 pens in the commercial meat meal-cod-liver oil-high corn oil series received 2, 4, and 8 parts, by weight, of cod-liver oil and 6, 4, and 0 parts, by weight, of corn oil, respectively. Viosterol was fed in the 3 pens of the high corn oil series at a level of 10, 20, and 40 D units, respectively, and in the 4 pens of the low corn oil series at a level of 20, 80, 160, and 320 D units, respectively. The meat meal used in compounding these diets was obtained in a single lot while that used in the commercial meat meal-cod-liver oil-direct sunlight pens consisted of several lots purchased at various times and places. The commercial meat meal containing liver was a specially prepared product.

TABLE III.

Incidence of short-boned embryos and chicks in eggs from birds which received diets supplemented by different types of meat meal and vitamin sources with or without access to direct sunlight.

| Supplement | | Embryos and chicks classified | | |
|---|---|---|---|---|
| | | Total | Short-boned individuals | |
| Kind | Parts by wt. | No. | No. | % |
| Commercial meat meal | 20 | 686 | 94 | 13.70 |
| Sunlight through glass | | | | |
| Cod-liver oil | 2-8 | | | |
| Corn oil | 6-0 | | | |
| Sunlight through glass | | 574 | 185 | 32.40 |
| Commercial meat meal | 20 | | | |
| Viosterol | (see text) | | | |
| High corn oil | 8 | | | |
| Sunlight through glass | | 337 | 132 | 39.17 |
| Commercial meat meal | 20 | | | |
| Viosterol | (see text) | | | |
| Low corn oil | 0.5 | | | |
| Commercial meat meal | 20 | 4,036 | 27 | 0.67 |
| Cod-liver oil | 2 | | | |
| Direct sunlight | | | | |
| Special commercial meat meal containing 10% liver | 20 | 9,994 | 6 | 0.06 |
| Cod-liver oil | 2 | | | |
| Direct sunlight | | | | |

The data in Table III show a very great difference in incidence of short-boned individuals in eggs from confined birds and those with access to direct sunlight. Part of the difference is probably due to composition or mode of preparation of the meat meals used. The data indicate, as do the data in Table 1, that liver contains some substance which reduces the incidence of the short-boned condition. The authors are not ready to advance an explanation for the difference between the sets of data from confined birds which received cod-liver oil and from those which received viosterol.

*Summary and Conclusions.* Abnormal embryos and chicks, with relatively short bones, were frequently produced in eggs laid by birds which received diets lacking in some factor or factors present in wheat germ, liver, and whey. The activity, amount or utilization of this factor or these factors was augmented by permitting the birds access to direct sunlight and green range.

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## 8170 P

## Reaction at Site of Injection of Mercurial Diuretics as Influenced by Theophylline.

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*From the Department of Therapeutics, New York University College of Medicine, New York.*

The comparative toxicity of mercurial preparations has usually been studied from the point of view of "minimal toxic dose" in the sense of causing death in a fixed period of time. The literature on this subject has been fully reviewed by Fourneau and Melville.[1] However, very little attention has been paid to the local reaction at the site of injection. The frequent occurrence of venous thrombosis and necrosis from the use of mercurial diuretics is of considerable importance. v. Issekutz and v. Vegh[2] combined a mercurial diuretic with theophylline and suggested that the latter, having weak acidic properties, neutralized the alkaline reaction (responsible for the pain) that is produced upon hydrolysis of the mercurial salt. This combination was introduced as novurit, but is known in this country as mercupurin.* Recently Fulton and Bryan[3] found that in animals both salyrgan and mercupurin produced definite necrotic lesions, but the former was more severe in extent and degree of reaction. They noted that there was a striking absence of slough formation when a mercurial salt, to which 5% theophylline was added, was injected subcutaneously.

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[1] Fourneau, E., and Melville, K. I., *J. Pharm. and Exp. Therap.*, 1931, **41**, 21.

[2] v. Issekutz, B., and v. Vegh, F., *Arch. f. exp. Path. u. Pharm.*, 1928, **138**, 245.

* Mercupurin is a complex mercurial salt differing from that of salyrgan in possessing a cyclopentane instead of a benzol ring. In addition, it has 5% theophylline, 3.5% of which is claimed to be chemically bound to it. Aminophylline is the double salt or mixture of theophylline and ethylene diamine. It has the advantage over theophylline of greater solubility.

[3] Fulton, N. M., and Bryan, A. H., personal communication.

We have compared the local action of mercurial diuretics with and without theophylline and have attempted to establish a quantitative relationship. The preparations used were mercupurin, the theophylline-free mercurial salt contained in mercupurin, salyrgan, salyrgan plus aminophylline (so that the theophylline content was equivalent to that of mercupurin) and as a control—aminophylline, physiological saline and distilled water.* Equivalent amounts of the drugs as regards mercurial content were used. The following series of experiments were performed:

(1) Doses of 0.05 cc. of various dilutions (undiluted to 1:100) of the drugs were injected intracutaneously into the abdominal wall of 12 rabbits. Each rabbit was given at the same experiment the various dilutions of at least 3 diuretics and the control drugs. It was noted that salyrgan and the mercurial salt of mercupurin free of theophylline produced in all dilutions an immediate well-circumscribed necrotic lesion, with absence of wheal formation and erythema. Mercupurin and the other mercurial salt, to which aminophylline had been added, produced in dilutions up to 1:10 a diffuse erythematous wheal. At the end of 24 hours the mercurial salts free of theophylline differed from the others in that they exhibited larger areas of necrosis and induration even with greater dilutions.

(2) The preparations undiluted were injected into the ears of 6 rabbits. The drugs lacking theophylline produced an immediate necrotic obliteration of all vessels in the vicinity and a striking absence of defense reaction. The lesions progressed, so that within 24 hours, a definite slough was evident. The drugs containing theophylline caused a diffuse wheal and vasodilatation with absence of slough formation.

(3) Histological studies of the areas injected and removed at various intervals from one-half to 48 hours revealed a striking absence of cellular response but extensive necrosis with salyrgan. Mercupurin caused necrosis limited to the needle tract and a cellular response resulting in abscess formation containing large numbers of polymorphonuclear leucocytes. These exhibited various stages of karryorhexis.

(4) Microinjections (6 for each drug) into the mesenteries of 12 mice by means of the Chamber's technique demonstrated the

high degree of toxic action upon mesenchymal tissue and blood vessels with those preparations not containing theophylline.

(5) It was found that intracutaneous injections in man (9 subjects) of 0.02 cc. of the drugs free of theophylline using dilution 1:10 produced a greater local reaction as regards extent, severity and duration than those preparations containing theophylline. In the dilution used the greatest reaction was the production of a small superficial slough.

It has been demonstrated that the presence of theophylline in combination with a mercurial diuretic definitely decreased the local toxic action. This was true not only for the animal experiments but also for man.

## 8171 C

## Blood Alcohol and Its Relation to Intoxication in Man.*

R. G. TURNER.

*From the Department of Medical Research, Wayne University, College of Medicine, Detroit.*

A method was described[1] for the detection of blood alcohol based on the Naville modification of the Nicloux method. It differs from the above modification in that the proteins are precipitated with the ordinary Folin-Wu reagents and the distillation is carried out with a modified ice condenser. When applied to blood distillates the method gives an average per cent error of 5.09 with a range of 0.3 to 15.0. The greater error is encountered in blood samples containing less than 10.5 mg. of alcohol. Schumm and Fleischman[2] showed that during the first hour the blood alcohol rose more rapidly than did the spinal fluid, but that during the decline of the blood alcohol the spinal fluid surpassed it and remained at a higher level. Abramson and Linde[3] showed that the alcohol content of the spinal fluid rose more slowly than that of the blood and reaches its maximum later. This maximum is lower than that reached by the blood. A previous communication[1] from this laboratory shows that intoxication in the dog is definite, if within one-half to two hours after ingestion the alcohol content of the blood measures 0.25%; at a blood alcohol concentration of 0.3% intoxication is marked and a concentration of 0.4 to 0.5% is accompanied by alcoholic stupor. Gettler and Freireich[4] published results indicating that there was a more constant relation between the alcohol content of the brain and spinal fluid than between the brain and blood.

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* The author wishes to extend his appreciation to Dr. David R. Clark for his cooperation in giving access to subjects brought into the hospital.

[1] Turner, R. G., *J. Pharm. and Exp. Therap.*, 1932, **44**, 305.

[2] Schumm, O., and Fleischman, R., *Deutsche Z. f. Neivenheilkunde*, 1913, **46**, 275.

[3] Abramson, L., and Linde, P., *Arch. Internat. de Pharmacodynamic et Ther.*, 1930, **39**, 325.

Newman and Mehrtens[5] in a report on 50 cases induced intoxication both by intravenous and oral route. They conclude that the alcohol content of the cerebral fluid closely follows that of the blood and correlates well with the symptoms of intoxication while that of the lumbar fluid shows a lag of about one hour and suggests that the better index is probably that of the blood.

In this paper the results of estimating blood alcohol in humans is reported. A total of 32 cases brought into Receiving Hospital, Detroit, for supposedly being drunk were tested. The blood was obtained from the cubital vein and the alcohol determined as previously described.

The degree of intoxication is indicated from the mode of action, appearance and speech as follows:

Intoxicated—Under the influence of liquor. Able to stand, but talk and walk not normal.

Markedly intoxicated—Able to stand but falls frequently.

Alcoholic Stupor—Not able to stand but would attempt to answer questions and attempt movements.

Coma—Unable to move or answer.

These are indicated by the blood alcohol concentration in the following manner:

| Blood Alcohol | % | |
|---|---|---|
| 0.1 | 0.2 | Not intoxicated |
| 0.21 | 0.3 | Intoxicated |
| 0.31 | 0.4 | Marked intoxication |
| 0.41 | 0.5 | Alcoholic stupor |
| 0.5 | 0.6 | Coma |

The results of this investigation show that 6.3% or 2 of the cases gave negative blood tests. One of these cases was later diagnosed as angina pectoris and hypertension. In the other case

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[4] Gettler, A. O., Freireich, A. W., *J. Biol. Chem.*, 1931, **92**, 1929.

[5] Newman, H. W., and Mehrtens, H. G., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 725.

## RESULTS

| Case and No. | Sex | Race | Age | Time blood taken | Condition | Blood alcohol % |
|---|---|---|---|---|---|---|
| E.H. 1 | Male | white | — | 10:30 P.M. | Intoxicated, not in stupor. Would not talk about what he drank or how much. | 0.321 |
| J.C. 2 | " | " | — | — | Slightly intoxicated, talkative but could walk with very little staggering. | 0.260 |
| F.M. 3 | " | " | — | — | Coma, cannot sit up or talk | 0.570 |
| R.F. 4 | " | " | 31 | 11:00 P.M. | Talkative but not intoxicated. Drank ½ pint of gin but did not say when. | 0.20 |
| C.M. 5 | " | " | 28 | 10:00 P.M. | Not intoxicated, talks moderateately. Drank 4 whiskeys. | 0.149 |
| L.R. 6 | " | " | 51 | 4:30 P.M. | Chronic alcoholic. In stupor. would not talk. Picked off street. | 0.440 |
| M.D. 7 | Female | colored | 32 | 3:30 P.M. | Picked off street in alcoholic stupor. Would not talk. | 0.220 |
| J.B. 8 | Male | white | 45 | 5:00 P.M. | Picked off street. Not in stupor. Marked intoxication. | 0.300 |
| M.M. 9 | Female | colored | 28 | 2:30 P.M. | Picked off street at 2:00 P.M. Talkative and crying. Could not answer questions intelligently. | 0.320 |
| F.C. 10 | Male | " | 38 | 10:45 A.M. | Liquor drank night before. Claims to have had none in morning. Hit by car. Talkative and appeared intoxicated. | 0.240 |
| E.J. 11 | Female | " | 31 | 10:10 A.M. | Throat cut. Last drink 3 hours previous. Talkative but not raving. Had 10-13 drinks of lightning. | 0.270 |
| F.C. 12 | Female | white | 25 | 12:50 P.M. | Struck by car. Very talkative and silly. Does not stagger, slightly unsteady. Drank wine at 10:30 A.M. | 0.250 |
| J.J. 13 | Male | colored | 32 | 10:30 A.M. | Last drink at 3 or 4 A.M. Had been hit on head by wife. Talks with sense. Does not stagger or appear intoxicated. Drank lightning. | 0.167 |
| T.P. 14 | " | white | 65 | 11:30 A.M. | Talkative, spry but unsteady on feet. Last drink 2 hours previous. | 0.230 |
| H.M. 15 | " | " | 60 | 2:15 P.M. | Taken to hospital at 1:40 P.M. unconscious. Still in stupor at 2:15 P.M. Refuses or cannot talk. | 0.275 |
| J.D. 16 | " | " | — | — | Unconscious. | 0.448 |
| J.E. 17 | " | " | — | — | Alcoholic stupor. Could not talk | 0.424 |
| D.M. 18 | Female | colored | 29 | 7:00 P.M. | On admittance slightly intoxicated. Could talk but refused. Drank pint of whiskey on empty stomach. | 0.260 |
| D.M. | " | " | " | 11:00 P.M. | Sober; could talk without difficulty. Would not state when she had taken liquor. | 0.140 |

RESULTS (Continued)

| Case and No. | Sex | Race | Age | Time blood taken | Condition | Blood alcohol % |
|---|---|---|---|---|---|---|
| J.D. 19 | Male | white | — | 11:05 P.M. | Markedly intoxicated. No information obtained regarding liquor. | 0.350 |
| J.S. 20 | ” | ” | — | 8:10 P.M. | Intoxicated. Could talk but refused to say anything. | 0.360 |
| J.M. 21 | ” | ” | — | — | No information regarding condition or time blood was taken. | 0.250 |
| W.S. 22 | ” | ” | 35 | 7:05 P.M. | Belligerent; talkative, crying. Fairly steady on feet. | 0.250 |
| W.A. 23 | ” | ” | 42 | 5:00 P.M. | Unconscious. No information obtained. | 0.300 |
| B.S. 24 | ” | ” | 35 | 3:30 P.M. | Rational. Had been drinking whiskey, gin, or anything continuously for 3 weeks. Staggered but not markedly intoxicated. | 0.390 |
| J.D. 25 | ” | ” | 37 | 7:15 P.M. | Alcoholic stupor. Regurgitated 15 minutes after admittance. | neg. |
| J.I. 26 | ” | ” | 42 | 4:30 P.M. | Alcoholic stupor. Regurgitated. Blood taken 10 minutes after admittance. | 0.250 |
| J.N. 27 | ” | ” | 51 | 4:45 P.M. | Fainted twice in County Bldg. Claimed loss of memory. Diagnosed later as angina pectoris and hypertension. | neg. |
| W.N. 28 | ” | ” | 49 | 7:45 P.M. | Fairly intoxicated; quite talkative. Had last drink one hour before. Says he is chronic. | 0.207 |
| R.H. 29 | Female | ” | 28 | 11:30 A.M. | Alcoholic stupor. Says she did not drink. Hysterical and raving at times. Would not answer questions. | 0.460 |
| C.L. 30 | Male | ” | — | 1:00 P.M. | Unconscious. Lacerated scalp and possible skull fracture. Accident case. | 0.330 |
| J.D. 31 | ” | ” | — | 11:50 P.M. | Unconscious. No information | 0.370 |
| J.D. 32 | ” | ” | 55 | 2:20 P.M. | Unconscious. After bath woke up but remained in stupor. Difficult to talk. | 0.450 |

no definite information was obtained. Four or 12.5% showed a blood alcohol of 0.1 to 0.2%. Of these, Case 4 stated that he drank ½ pint of gin but would not say when. Case 13 had last drink between 3 and 4 A. M. At 10:30 A. M., the time the blood was taken, he appeared normal. Previously he had been hit on the head by his wife. Thirty-seven and five-tenths per cent of the cases appeared intoxicated and the blood alcohol level was between 0.21 and 0.3%. In this class 41.6% (cases 7, 15, 26, 23 and 8) showed more intoxication than the test revealed. Marked intoxication, 0.31 to 0.4% blood alcohol level, was found in 25% of the cases. Of these cases two were unconscious. Case 30 is explainable because of a lacerated scalp and possible skull fracture. No information was obtained in Case 32.

Alcoholic stupor was observed in 15.6% with blood alcohol between 0.41 and 0.5%. Of these cases two were unconscious. Case 32 was unconscious but woke up after a bath.

The cases showing a blood alcohol from 0.5 to 0.6% amounted to 3.1%. The above result for non-intoxication 0.1 to 0.2% blood alcohol apparently is greater than the amount indicated by Heise,[6] who believes loss of efficiency and judgment occurs even when small amounts of alcohol, 0.02%, accumulate in the blood and urine, although the individual can still walk and talk. It is shown from the results given that in all the stages of intoxication a small percentage of the cases always show more signs of intoxication than the blood test actually reveals. This we believe to be due to accidents, complications, taking hypnotics or other sleep producing substances previous to the consumption of alcoholic drinks. Experiments have been conducted on dogs to partially prove the latter point and the results will be reported in another communication.

*Summary.* In general, intoxication is not noticeable in the human until the blood alcohol concentration is greater than 0.2%; from 0.31 to 0.4% there is marked intoxication; alcoholic stupor is definite between 0.41 and 0.5%; above 0.5% coma or death may result.

### 8172 C

## Effect of Total Pneumonectomy on Position of the Esophagus.*

ALFRED BLALOCK.

*From the Department of Surgery, Vanderbilt University.*

The increasing number of instances in which the total removal of one lung in man is being performed for tumor or bronchiectasis has created renewed interest in the effects of this procedure. The experiments reported here deal with the effects of total pneumonectomy on the position of the esophagus of the dog, but it does not follow necessarily that similar alterations occur in the human.

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[6] Heise, H. A., *J. A. M. A.*, 1934, **103**, 739.

* Aided by a grant to Vanderbilt University from the Division of Medical Sciences of the Rockefeller Foundation.

A complete removal of either the right or left lung was performed in 4 dogs. Anesthesia was produced by the administration of ether through a tracheal catheter which was connected to a positive pressure device. The operation was performed through an

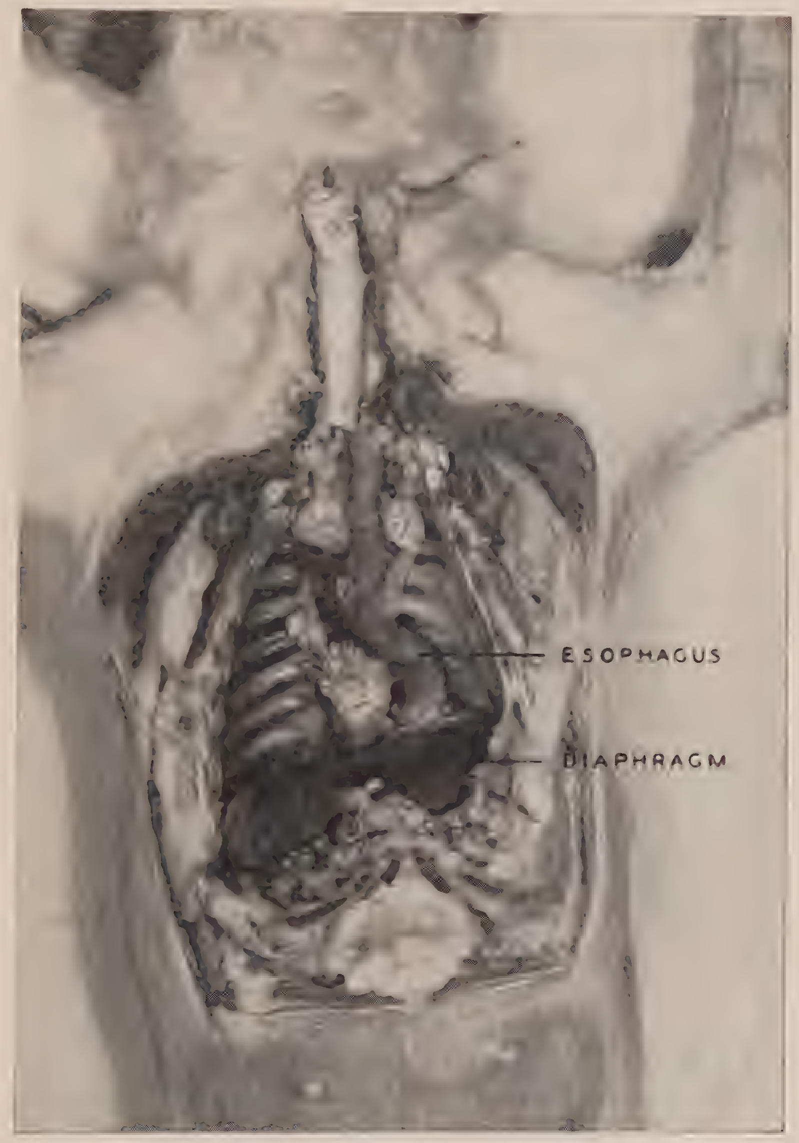

FIG. 1.
Showing the deviation of the esophagus to the left following the total removal of the left lung. Trachea in midline. Diaphragm not paralyzed.

intercostal incision and the chest was closed tightly following the removal of the lung. When the termination of the experiment was decided upon, the animal was bled to death, the remaining blood was washed out by injecting salt solution intravenously and this was followed by the introduction of 10% formalin. The chest cavity

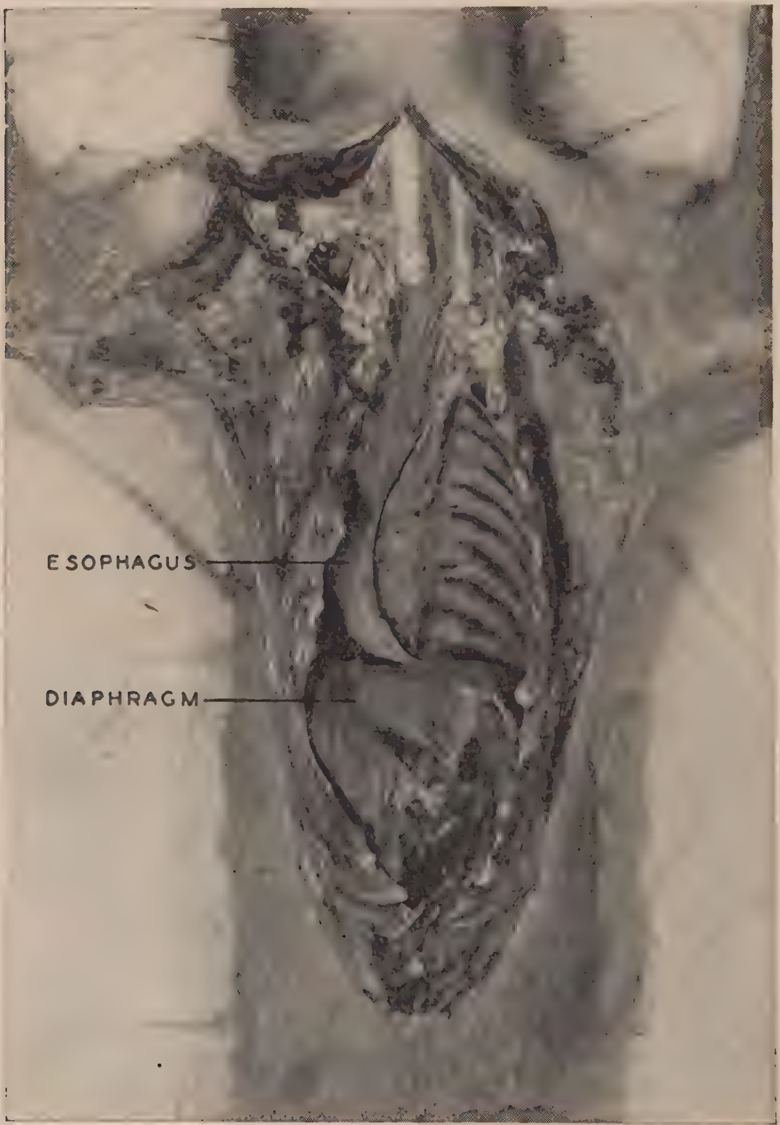

FIG. 2.
Showing the deviation of the esophagus to the right following the total removal of the right lung. Diaphragm not paralyzed.

was then opened and the position of the esophagus was determined.

The first animal was killed 10 months after the left lung had been removed. The left pleural cavity seemed to be somewhat smaller than the right and the left side of the diaphragm was slightly higher than the right. The filling of the chest appeared to be due in the main to an increase in size of the right lowermost lobe. The lower part of the esophagus occupied a position far to the left of the midline. This is shown in Fig. 1. There was no constriction of the esophagus and no ulceration was noted.

The second animal was killed 5 months following the total removal of the left lung. The findings were similar to those observed in the first experiment.

The third animal appeared to have distemper 5 weeks following the total removal of the right lung. Death was caused painlessly and the usual procedure carried out. The esophagus was found to be markedly deviated to the right. The position is shown in Fig. 2. The 2 sides of the diaphragm were at approximately the same levels.

In the fourth experiment, the left lung was removed and the left phrenic nerve was paralyzed. Fifteen weeks later it was observed that the left diaphragm was very much higher than the right and that the esophagus occupied essentially its normal position.

*Summary.* Total pneumonectomy in dogs, without an associated paralysis of the diaphragm, was found to be followed by a marked deviation of the esophagus to the side from which the lung had been removed.

### 8173 C

## Reactivation of Ammonia-Inactivated Complement by Leucocytes.

ELIZABETH MALTANER. (Introduced by Augustus Wadsworth.)

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From the earliest work on the lytic action of serum, the cells of the tissues, particularly the phagocytes, have been considered a source of complement. However, convincing proof is still lacking that these cells either contain or liberate active complement. The possibility that they may contribute substances inactive alone but nevertheless essential to complete complement action has received little consideration.

It seemed of interest to determine whether or not a suspension of leucocytes would contribute complementing substances, either heat-labile or heat-stable, which, like heated serum,[1] might restore the hemolytic activity of complement made inactive by ammonia.

Fresh guinea pig serum was inactivated with half its volume of N/5 ammonium hydroxide at 37°C. for 1½ hours and then neutralized to pH 7.6 with N/5 hydrochloric acid. The final dilution was 1:2.

Leucocytes from guinea pig blood were collected in strata and washed in physiological salt solution by centrifugalization at low speed, according to the technic of Wadsworth and Hoppe.[2] They were washed, however, not once but 5 times. Sufficient salt solution was used in washing so that the dilution of plasma in the final suspension of leucocytes was at least 1:200,000. Also, in order to compensate for the unavoidable mutilation of the cells during the additional washings, the leucocytes from 10 cc. of blood were suspended in 0.5 cc., rather than in 1 cc., of salt solution.

In order to control the reactivating effect of traces of plasma which might be retained in the preparation of the leucocyte suspension, a portion of the supernatant fluid from which these cells had been separated was diluted to the same degree as were the leucocytes in the process of washing, and tested in parallel with them for its reactivating effect. For comparison, the reactivating properties of very small amounts of heated serum were also determined.

Tests for hemolytic action were made as indicated in the protocol. A 5% suspension of sheep cells sensitized with 2 units of amboceptor was used. Readings were made by comparison with color standards.[3]

The leucocyte suspension, unheated or heated for one-half hour at 55°C., completely, or nearly completely, reactivated the ammonia-inactivated complement in 2 hours at 37°C. in the water bath.* The

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[1] Gordon, John, Whitehead, H. R., and Wormall, Arthur, *Biochem. J.*, 1926, **20**, 1028.

[2] Wadsworth, A. B., and Hoppe, E. N., *J. Immunol.*, 1921, **6**, 399.

[3] Gilbert, Ruth, Kelley, M. F., and Moore, A. C., *J. Lab. and Clin. Med.*, 1925, **10**, 552.

* The complementary effect of leucocytes may account for the opsonic action which has been observed with ammonia-treated serum.[4–6]

[4] Gordon, John, and Thompson, F. C., *Brit. J. Exp. Path.*, 1935, **16**, 101.

[5] Gordon, John, Whitehead, H. R., and Wormall, Arthur, *J. Path. and Bact.*, 1929, **32**, 57.

[6] Gordon, John, Whitehead, H. R., and Wormall, Arthur, *Biochem. J.*, 1926, **20**, 1044.

TABLE I.

Reactivation of Ammonia-inactivated Complement by Diluted Plasma or Serum and by Washed Leucocytes.

| Reagents | Dilution | Amount tested, in cubic centimeters or in drops | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ammonia-inactivated complement | 1:2 | 0.2 | 0.25 | 0.2 | 0.25 | 0.2 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | | | | |
| Guinea-pig serum heated ½ hr. 55° C. | 1:50 | | | | | | | 0.1 | | | | | | | | | | |
| | 1:400 | | | | | | | | 0.1 | | | | | | | | | |
| | 1:1600 | | | | | | | | | 0.1 | | | | | | | | |
| | 1:6400 | | | | | | | | | | 0.1 | | | | | | | |
| Plasma from first centrifugalization of cells | 1:5000 | | | | | | | | | | | 0.1 | | | | | | |
| | 1:50,000 | | | | | | | | | | | | 0.1 | | | | | |
| | 1:200,000 | | | | | | | | | | | | | 0.1 | | | | |
| Ammonia-inactivated complement heated ½ hr. 55° C. | 1:2 | | | | | | | | | | | | | | 0.25 | | | |
| Untreated complement | 1:2 | | | | | | | | | | | | | | | 0.25 | 0.25 | |
| Leucocyte suspension | | | | | | | | | | | | | | | | | | |
| Unheated | | | | 1 gt | 1 gt | | | | | | | | | | 1 gt | | 1 gt | 4 gtt |
| Heated ½ hr. 55° C. | | | | | | 1 gt | 1 gt | | | | | | | | | | | |
| | | % hemolysis in water bath, 37° C. | | | | | | | | | | | | | | | | |
| After 15 min. | | 0 | 0 | 10 | 25— | 25+ | 50 | 90+ | 35 | 0 | 0 | 10 | 0 | 0 | 0 | 100 | 100 | 0 |
| ,, 2 hr. | | 0 | 2 | 75+ | 90+ | 100 | 100 | 100 | 90+ | 30 | 2 | 50 | 0 | 0 | 0 | 100 | 100 | 0 |

Sensitized cells were used in 0.4 cc. amounts, and the volume in all tubes was equalized with physiological salt solution.

heated suspension was even more active than the unheated. Plasma diluted to the same extent as were the leucocytes in washing—namely, 1:200,000—had no reactivating effect; in an amount 40 times as great, a 1:5000 dilution, it partially restored the activity of ammonia-treated serum; 50% hemolysis was obtained in 2 hours at 37°C. Heated serum in a 1:400 dilution reactivated to about the same degree as the unheated leucocyte suspension; the limit of its activity was reached in about a 1:6400 dilution.

After heating for one-half hour at 55°C., the ammonia-inactivated complement was not reactivated by the leucocyte suspension; the latter, in 4 times the amount used in the other tests, had no hemolytic action alone.

Thus the leucocytes contain or convey complementary substances essential to the hemolytic activity of ammonia-treated serum. Further study may determine more clearly whether the leucocytes are active *per se,* or through the absorption of minute quantities of serum or plasma.

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## 8174 C

## Some Analyses of Thyroglobulin.

ABRAHAM WHITE.

*From the Department of Physiological Chemistry, Yale University, New Haven.*

In view of the physiological importance of thyroglobulin, it is somewhat surprising that little information has been made available concerning its amino acid composition. Until recently, the investigation of Eckstein[1] represented the only study, by more recent methods of protein analysis, of the distribution of the more important amino acids of thyroglobulin. The basic amino acids yielded on hydrolysis of this protein have been recently determined in this laboratory[2] by isolation procedures, and the results compared with those of Eckstein. Additional data have now been obtained regarding some of the other amino acids of thyroglobulin and are being presented inasmuch as further chemical investigation of this protein is not contemplated at the present time. A portion of these analyses supplement information already available in the literature;

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[1] Eckstein, H. C., *J. Biol. Chem.*, 1926, **67**, 601.

[2] White, A., and Gordon, W. G., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 354.

others represent the first determinations of certain of the amino acids of thyroglobulin.

The preparation used for this analytical work has been previously described.[2] Glutamic acid and aspartic acid were determined by the isolation procedure of Jones and Moeller.[3] The tyrosine and tryptophane determinations were conducted as described by Folin and Ciocalteau.[4] Proline was determined by utilizing the fractionation technique recently described by Jukes.[5] The percentage of proline is calculated from the weight of the analytically pure cadmium chloride salt, isolated as recommended by Kapfhammer and Eck.[6] Cystine analyses were conducted by 2 methods, the colorimetric procedure of Folin and Marenzi,[7] and the cysteine cuprous mercaptide method of Vickery and White.[8] Cystine values found by the 2 methods are in good agreement. The results of the various determinations, expressed as percentages of the ash- and moisture-free protein, are presented in Table I. Results already reported[2] are also included to provide the complete data which have been obtained for this protein in this laboratory.

TABLE I.
Some Analyses of Thyroglobulin.

| | Analysis I<br>% | Analysis II<br>% | Average<br>% | Results of Eckstein[1]<br>% |
|---|---|---|---|---|
| Total Nitrogen | | | 15.58 | |
| '' Sulfur | | | 1.46 | |
| '' Iodine | | | 0.75 | |
| Glutamic Acid | 6.61 | 6.52 | 6.56 | |
| Aspartic '' | 1.76 | 1.43 | 1.59 | |
| Tyrosine | 3.15 | 3.18 | 3.17 | 5.45 |
| Tryptophane | 1.72 | 1.88 | 1.80 | 2.15 |
| Cystine | | | | 1.55 |
| Folin-Marenzi method | 2.06 | 2.09 | 2.07 | |
| Vickery-White '' | 2.00 | 2.05 | 2.03 | |
| Proline | 4.34 | 4.61 | 4.47 | |
| Histidine | | | 0.62 | |
| Arginine | | | 8.22 | |
| Lysine | | | 1.93 | |

The cystine, tyrosine and tryptophane values found in the present investigation do not agree well with those reported by Eckstein.[1] However, the latter investigator employed the methods described earlier by Folin and Looney;[9] recent improvements in the procedures of estimating these amino acids have frequently given results which are at variance with those obtained by the original technique of Folin and Looney.† The glutamic acid, aspartic acid and proline values are, so far as it has been possible to determine from the literature, the first recorded quantitative determinations of the amounts of these amino acids yielded by thyroglobulin.

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[3] Jones, D. B., and Moeller, O., *J. Biol. Chem.*, 1928, **79**, 429.

[4] Folin, O., and Ciocalteau, V., *J. Biol. Chem.*, 1927, **73**, 627.

[5] Jukes, T. H., *J. Biol. Chem.*, 1933, **103**, 425.

[6] Kapfhammer, J., and Eck, R., *Z. physiol. Chem.*, 1927, **170**, 294.

[7] Folin, O., and Marenzi, A. D., *J. Biol. Chem.*, 1929, **83**, 103.

[8] Vickery, H. B., and White, A., *J. Biol. Chem.*, 1932-33, **99**, 701.

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## 8175 P

## Experimental Rabies in White Mice. Studies on Passive Immunization I.*

ANSON HOYT, ROY T. FISK, and FREDERICK J. MOORE. (Introduced by John F. Kessel.)

*From the Department of Bacteriology, School of Medicine, University of Southern California and the Los Angeles County Hospital, Los Angeles.*

The subject of passive immunization against rabies has received relatively little attention, even though Fermi,[1] Pfeiler[2] and others have reported striking experimental demonstrations of the efficacy of anti-rabic serums. In the current report, which summarizes 12 protection tests performed in the past 2 years, the protective properties of such serums have been studied using white mice as experimental animals.

A fixed virus strain, obtained through the courtesy of the Cutter Laboratories, was passed through rabbits and preserved at ice-box temperature, either fresh or in 50% glycerine, for periods which did not exceed 2 weeks. Details of preparation varied, but in effect the virus was ground to an initial dilution of 1/20 in normal saline and centrifuged 10 minutes at 2200 R.P.M. The supernatant fluid was then filtered through "Whatman No. 1" paper and final dilutions of from 1/100 to 1/800 were made. The virus was injected intracerebrally, at first in amounts proportional to the body weights of the mice (0.005 cc./gm. or 0.001 cc./gm.), later in a constant dose of 0.02 cc.

The serums were prepared by hyperimmunizing rabbits and goats with either 1/10 fresh fixed virus or 1/20 fixed virus preserved with ½% phenol. Serums were developed which would neutralize equal volumes of 1/200 fixed virus prepared as above to a final titer of 1/256, when the mixtures were incubated at 37° C. for 2 hours and tested by intracerebral mouse injection. Corresponding normal serums showed no *in vitro* neutralizing properties. The serums were filtered and inactivated before use.

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[9] Folin, O., and Looney, J. M., *J. Biol. Chem.*, 1922, **51**, 421.

† For examples and amplification of this statement, see references 4 and 7.

* This work was made possible through a grant from Mrs. John Sherman Hoyt.

[1] Fermi, C., *Cent. f. Bakt., Parasit. u. Infektskr.*, Orig., 1909, **52**, 576.

[2] Pfeiler, W., *Berliner Tierarzt. Wochenschr.*, 1913, **29**, 269.

In the experiments reported the serums were injected intraperitoneally, the first dose being given at intervals ranging from 48 hours to 1 hour before the administration of virus. Doses for each serum injection varied from .05 cc./gm. body weight to .15 cc./gm. Serum was usually given only once, but at times as many as 2 subsequent injections were made, the maximum total of serum given to any mouse being .45 cc./gm. in a period of 5 days. Control mice, either untreated or receiving corresponding amounts of normal serum, were included in all experiments. Titrations of the intracerebral infectivity of the virus and of the neutralizing titer of the serum against the virus employed were run simultaneously with most experiments. The virus often infected in dilutions as high as 1/6400. All mice were observed for symptoms at 12-hour intervals.

Any mouse that remained symptom-free for more than 2 weeks was classified as a survival. The results† of the separate tests evidence a reliability sufficient so that they may be assumed to indicate a normal statistical expectancy. Their characteristics have been examined and it has been determined that the separate tests can be combined by totaling and, considered, in the aggregate, as one experiment, which includes 155 treated and 198 control mice.

The mice contracting rabies in the different tests were also considered. It was found that the mean incubation period of each treated group was longer than that of its control group. An inspection of the corresponding standard deviations showed that these variations are significant.

From a study of the results of the experiments it is obvious that some evidence of passive protection, apparently due to specific serum therapy, was found in each test. Such protection was demonstrable in one or both of the following ways: (a) A higher percentage of treated mice (39%) survived than of the corresponding controls (12%). (b) A longer mean incubation period was shown by the treated mice that contracted rabies than by their corresponding controls. We are continuing this study in an effort to evaluate the maximum efficacy of anti-rabic serum.

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† We are indebted to Dr. Thurston H. Ross of the Department of Commerce, University of Southern California, for a careful statistical analysis of our data.

## 8176 P

# Influence of Amino Acids and of Protein Diet Upon Creatine Content of the Myocardium.

EATON M. MACKAY AND RICHARD H. BARNES.

*From the Scripps Metabolic Clinic, La Jolla, California.*

Beard and Barnes[1] have reported that the feeding of various amino acids and proteins resulted in an increase in the creatine (total creatinine) concentration in skeletal muscle tissue. Preparatory to a study of the influence of dietary ingredients upon the reduction in the creatine content of heart muscle, produced by experimental thyrotoxicosis[2] and other procedures, the influence of feeding various amino acids and of the protein intake upon the creatine content of the myocardium was observed.

Adult rats were fed the special diets for a period of 2 weeks. The 3 casein diets all contained 4% of the Osborne and Mendel salt mixture, 10% of brewers yeast, 10% cod liver oil and 15% lard. In order, they contained 6, 20 and 55% of casein, and 55, 41 and 6% respectively of cornstarch. The 20% casein diet served as the control diet and for the addition of amino acids. Muscle creatine was determined by the method of Rose, Helmer and Chanutin.[3]

Table I shows that the higher the protein content of the diet the

TABLE I.
Creatine Concentration.

| No. of Rats | Sex | Body Weight | Myocardium Mg. % Low | Myocardium Mg. % High | Myocardium Mg. % Aver. | Skeletal Muscle Mg. % Low | Skeletal Muscle Mg. % High | Skeletal Muscle Mg. % Aver. | Diet |
|---|---|---|---|---|---|---|---|---|---|
| 4 | ♂ | 314 | 160 | 184 | 169 | 375 | 417 | 397 | Control |
| 4 | ♀ | 237 | 187 | 200 | 192 | 372 | 403 | 382 | " |
| 3 | ♂ | 347 | 162 | 200 | 179 | 357 | 388 | 374 | 5% Glycine |
| 3 | ♀ | 227 | 182 | 192 | 187 | 366 | 440 | 405 | 5% " |
| 7 | ♂ | 212 | 177 | 232 | 208 | 393 | 452 | 420 | 10% " |
| 5 | ♀ | 155 | 205 | 235 | 220 | 389 | 445 | 409 | 10% " |
| 3 | ♂ | 199 | 189 | 211 | 197 | 408 | 446 | 428 | 10% Alanine |
| 4 | ♀ | 149 | 196 | 225 | 209 | 413 | 481 | 448 | 10% " |
| 4 | ♂ | 221 | 165 | 197 | 180 | 388 | 430 | 408 | 10% Glutamic Acid |
| 2 | ♀ | 170 | 190 | 207 | 198 | 411 | 442 | 426 | 10% " " |
| 6 | ♂ | 226 | 185 | 227 | 212 | 420 | 440 | 434 | 6% Casein Diet |
| 6 | ♀ | 185 | 177 | 264 | 207 | 417 | 474 | 439 | 6% " " |
| 6 | ♂ | 357 | 175 | 198 | 181 | 388 | 417 | 401 | 20% " " |
| 6 | ♀ | 215 | 169 | 195 | 183 | 372 | 440 | 401 | 20% " " |
| 7 | ♂ | 205 | 164 | 188 | 175 | 379 | 415 | 405 | 55% " " |
| 7 | ♀ | 166 | 163 | 193 | 176 | 374 | 435 | 403 | 55% " " |

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[1] Beard, H. H., and Barnes, B. O., *J. Biol. Chem.*, 1931-32, **94**, 49.

[2] Cowan, D. W., *Am. J. Physiol.*, 1934, **109**, 312.

[3] Rose, W. C., Helmer, O. M., and Chanutin, A., *J. Biol. Chem.*, 1927, **75**, 543.

lower the concentration of creatine in the myocardium. This is surprising and may be due to the variable carbohydrate intake, a point which is being examined. The addition of glycine or dl-alanine to the diet definitely raised the amount of creatine in the heart muscle, d-glutamic acid was not so effective. The relative effects of the various amino acids, particularly over short periods of time in the same manner as used by Beard and Barnes[1] for skeletal muscle, should be determined.

## 8177 C

## Sodium Propyl-methyl-carbinyl Allyl Barbiturate, a Short Acting Hypnotic.

EDWARD E. SWANSON. (Introduced by K. K. Chen.)

*From the Lilly Research Laboratories, Indianapolis.*

Fitch, Waters, and Tatum[1] in their extensive work on barbituric acid hypnotics emphasized the importance of employing short acting members for surgical procedures. Among the different derivatives synthesized by Shonle and his associates,[2, 3, 4] the sodium salt of propyl-methyl-carbinyl allyl barbituric acid appears to have the desirable promptness and brevity of action. The same compound has been prepared by Tabern and Volwiler.[5]

By following Eddy's scheme of recording results,[6] it was found that in rats by intraperitoneal injection the minimal anesthetic dose (M.A.D.) was 40 mg. and the minimal lethal dose (M.L.D.) 110 mg., per kg. Twenty mg. per kg. by the same route of administration were sufficient to induce sleep. These animals, 220 in number, weighed on the average 94 gm. In dogs by intravenous injection of a 5% solution at the rate of 1 cc. per minute, the M.A.D. was ascertained to be 25 mg. and the M.L.D. 50 mg., per kg. By mouth in the same species of animal the M.A.D. was determined to be 35 mg. and the M.L.D. 90 mg., per kg. A total of 83 dogs were used.

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[1] Fitch, R. H., Waters, R. M., and Tatum, A. L., *Am. J. Surg.*, 1930, **9**, 110.

[2] Shonle, H. A., Keltch, A. K., and Swanson, E. E., *J. Am. Chem. Soc.*, 1930, **52**, 2440.

[3] Shonle, H. A., and Kleiderer, E. C., *J. Am. Chem. Soc.*, 1934, **56**, 2489.

[4] Shonle, H. A., *J. Am. Chem. Soc.*, 1934, **56**, 2490.

[5] Tabern, D. L., and Volwiler, E. H., *J. Am. Chem. Soc.*, 1934, **56**, 1139.

[6] Eddy, N. B., *J. Pharm. and Exp. Therap.*, 1928, **33**, 43.

TABLE I.

Comparison of Sodium Propyl-methyl-carbinyl Allyl Barbiturate, Pentobarbital Sodium, and Sodium Amytal in Dogs.

| | | M.A.D. by vein | | | | M.A.D. by mouth | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Drug | Dogs used | Amt. required mg. per kg. | Duration of anesthesia min. | Time necessary for complete recovery hr. | min. | Amt. required mg. per kg. | Appearance of ataxia after injection min. | Duration of anesthesia min. | Time necessary for complete recovery hr. | min. |
| Sodium Propyl-methyl-carbinyl Allyl Barbiturate | 5 | 25 | 70 | 7 | 50 | 35 | 4 | 75 | 12 | 10 |
| | | | 70 | 7 | 20 | | 4 | 140 | 12 | 45 |
| | | | 50 | 7 | 10 | | 3 | 160 | 12 | 43 |
| | | | 80 | 7 | 35 | | 3 | 120 | 12 | 10 |
| | | | 50 | 7 | | | 5 | 140 | 12 | 15 |
| Aver. | | | 64 | 7 | 23 | | | 127 | 12 | 24 |
| Pentobarbital Sodium | 6 | 25 | 35 | 9 | | 40 | 5 | 140 | 17 | |
| | | | 30 | 8 | 30 | | 5 | 110 | 18 | |
| | | | 65 | 8 | 30 | | 8 | 135 | 15 | |
| | | | 45 | 7 | 30 | | 5 | 145 | 12 | 30 |
| | | | 35 | 8 | 30 | | 5 | 130 | 13 | 30 |
| | | | 45 | 9 | | | 8 | 60 | 14 | |
| Aver. | | | 42 | 8 | 30 | | | 120 | 15 | |
| Sodium Amytal | 6 | 45 | 65 | 16 | | 70 | 8 | 135 | 22 | |
| | | | 65 | 15 | 30 | | 8 | 150 | 22 | |
| | | | 50 | 14 | | | 10 | 180 | 22 | |
| | | | 55 | 16 | 30 | | 8 | 150 | 24 | |
| | | | 65 | 16 | | | 8 | 175 | 24 | |
| | | | 60 | 14 | | | 10 | 105 | 20 | |
| Aver. | | | 60 | 15 | 20 | | | 149 | 22 | 20 |

A comparison of the onset and duration of action of sodium propyl-methyl-carbinyl allyl barbiturate in dogs with those of sodium amytal and pentobarbital sodium is shown in Table I. It will be noted that when given by mouth the appearance of ataxia occurred slightly sooner with the new barbituric acid derivative. Its duration of anesthesia was, on the average, longer than that with pentobarbital sodium and comparable to that with sodium amytal. However, the time for complete recovery after the administration of sodium propyl-methyl-carbinyl allyl barbiturate was approximately half that with sodium amytal, and slightly shorter than that with pentobarbital sodium.

Similar to sodium amytal and pentobarbital sodium, the new compound by intravenous injection proved to protect rabbits from 4 but not 5 M.L.D.'s of strychnine or cocaine—34 animals being employed for this purpose. Its preanesthetic value in rats was found to be 25% of the M.L.D. according to the method of Barlow and his coworkers.[7] In this case, the drug was given subcutaneously, and the subcutaneous M.L.D was determined to be 140 mg. per kg. In 5 dogs which were repeatedly injected by vein with anesthetic doses, every other day for 8 days, there was no loss of hypnotic and anesthetic effect. Furthermore, it required no more than the M.L.D. (50 mg. per kg.) to kill the same animals. Obviously, no tolerance was developed by repeated administration. The new hypnotic when injected intravenously in anesthetic doses did not appear to be excreted in the urine. Like all other members of the same group, sodium propyl-methyl-carbinyl allyl barbiturate causes lowering of blood pressure and depression of respiration, especially if given rapidly by vein. The body temperature may also fall slightly. No inhibition of the vagus in dogs was observed when anesthetic doses were employed. In this respect, sodium propyl-methyl-carbinyl allyl barbiturate is different from sodium amytal but similar to pentobarbital sodium.

Clinical investigations have been carried out by Doctors G. F. Kempf and L. G. Zerfas, and separate reports will be made by them elsewhere. In general, their results bear out the essential points enumerated above, particularly with reference to the promptness and brevity of action.

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[7] Stormont, M. F., Lampe, I., and Barlow, O. W., *J. Pharm. and Exp. Therap.*, 1930, **39**, 165.

### 8178 P

# Method for Concentrating the Gonadotropic Activity in Pregnancy Urine.*

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*From the Zoological Laboratory, University of Wisconsin.*

For the past several years we have employed a procedure for concentrating the gonadotropic substance in the urine of pregnant women which has yielded an excellent extract for general physiological purposes. The method is not difficult to carry out, is quantitative and the product is non-toxic even though administered in massive doses. The procedure is based on the fact that the gonadotropic material from pregnancy urine is precipitated by tannic acid, and by treating the tannic precipitate with pyridine the active principle is again set free from the tannic acid and becomes water soluble.

An aqueous concentrate of tannic acid is added to the urine until no further precipitation takes place and, after standing in the cold for several hours, the precipitate is collected by suction filtration, washed with acetone and powdered. The powder may be stored as such and has been kept for as long as 3 years without detectable loss in activity. For further purification, the powder is extracted 8 to 10 hours with constant stirring with 50% aqueous pyridine (200 cc. per 10 gm.—the equivalent of about 5 liters of the original urine). The residue is removed by centrifuging and re-extracted with a like amount of pyridine solution for the same length of time. The two solutions are united and evaporated in a warm current of air (40°C.) or precipitated by pouring into 10 volumes of acetone, adding a small amount of saturated sodium chloride or sodium acetate solution. The residue is then extracted 4 hours with from 10 to 15 cc. distilled water 5 different times, centrifuging each time (cooling the solution to freezing just before centrifuging aids in throwing down the precipitate). All of the solutions are united and the activity precipitated by adding 10 volumes of acetone. The precipitate, which contains the activity, is removed by centrifuging and again extracted 5 times with from 3 to 5 cc. of water each time and again precipitated with 10 volumes of acetone. The resultant precipitate is readily soluble in a volume of water representing a 500-fold concentration of the original urine. As much as 6 cc. of the extract (3 l. urine equivalents), administered within 3 days into immature rats and 2 cc. per day injected intravenously into sexually immature monkeys for 10 days, is not toxic.

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* Aided by a grant from the National Research Council, Committee on Problems of Sex, administered by F. L. Hisaw.

TABLE I.

The Ovarian Weights of Immature Rats, 22 Days Old, after Injection of Definite Amounts of 3 Different Urine Samples, Variously Treated. The Vaginæ of All Test Rats Were Open at the Time of Autopsy.

| Urine sample | Amt. | Whole urine | Ov. wts. in mg. after treatment with Tan. Ppt. | Extr. | Diluted 1:4 Nor. Ur. | Dil. 1:4 Boiled Ur. | Dil. 1:250 Boiled Ur. |
|---|---|---|---|---|---|---|---|
| 1 | 2 cc. | 27 | 28 | 26.4 | | | |
| | | 26 | 29 | 25.5 | | | |
| | | | 25 | | | | |
| 2 | 1 cc. | 22 | 21 | 20 | | | |
| | | 20 | 21.5 | 23 | | | |
| 3 | 2 cc. | 23 | 27.5 | | 23 | 27 | |
| | | 34 | 28 | | 28.5 | 24.5 | |
| | | 21.5 | 25.5 | | 29 | 26.5 | |
| | | 23.5 | | | | | |
| | | 22.5 | | | | | |
| | .5 cc.* | 16 | 20.5 | 17.5 | 18 | 19 | 17 |
| | | 18 | 16 | 16 | 19.5 | 16.5 | 15.5 |
| | | 16.5 | 16.5 | 17 | 17.5 | 18.5 | 18 |
| | | | | | | | 16 |

* Ovarian weights of uninjected controls 11 to 14 mg.

Table I presents the resultant weights of immature rat ovaries after administration of 3 different urine samples. The urines were first standardized by direct injection after extracting with ether to remove the oestrin. They were then precipitated with the tannic acid and both the crude precipitates and purified extracts were assayed. The method is quantitative since the purified extract in comparable doses is as active as the original urine. To further test the completeness of the precipitation and of the extraction, the supernatant urine after tannic acid treatment was precipitated with 10 volumes of acetone and this precipitate in 250 cc. equivalent amounts induced no stimulation in immature female rats; likewise the pyridine and water insoluble fractions in the same equivalent amounts were without effect.

To find whether the method would give quantitative results for less concentrated solutions, aliquot portions of pregnancy urine (Sample 3) were diluted 4 times with normal and boiled male urine, precipitated, and extracted in the usual manner. Another portion of the pregnancy urine was diluted 250 times with boiled male urine and extracted. It can be seen in the table that total recovery of gonadotropic activity was accomplished in each instance.

### 8179 C

## Lack of Effect of Theelin upon Somatogenic, Thyreotropic, and Adrenotropic Activity of Hypophysis.

HARRIS B. SHUMACKER, JR., AND AUSTIN LAMONT. (Introduced by W. M. Firor.)

*From the Surgical Hunterian Laboratory of the Johns Hopkins Medical School.*

It has been well established that rats treated with theelin undergo a diminution in the sex-stimulating power of the hypophysis.[1, 2, 3] Nelson[4] has recently demonstrated changes in the basophilic cells of the hypophyses of rats following injection of theelin which he interprets as indicating inhibition of secretory activity. Similar findings are recorded by Halpern and D'Amour.[5] The same changes are described in the pituitaries of rats treated with pregnancy-urine extracts,[4, 6, 7, 8] and these alterations are interpreted as secondarily due to increased secretion of the gonadal hormone.[4, 6, 7, 9] There is some disagreement as to the changes in the acidophilic cells. Nelson[4] records a decrease in the number of normal cells, with degranulated forms. Severinghaus[7] finds little change in immature females, a reduction in number, size, and staining reaction in adult females and an increase in number, size, and staining reaction in adult males. Wolfe[8] describes an increase in infantile females. Barnes, Reagan, and Nelson[10] have demonstrated that in depancreatized dogs the injection of theelin has a similar effect to that of total hypophysectomy. Nelson and Overholszer[11] have recently reported confirmatory experiments in depancreatized monkeys.

Since the initial work indicates very strongly that the administration of theelin has an inhibitory effect upon the histological pic-

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[1] Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 702.

[2] Moore, C. R., and Price, D., *Am. J. Anat.*, 1932, **50**, 13.

[3] Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J., *Endocrinol.*, 1932, **16**, 655.

[4] Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 452.

[5] Halpern, S. R., and D'Amour, F. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 108.

[6] Leonard, S. L., *Anat. Rec.*, 1933, **57**, 45.

[7] Severinghaus, A. E., *Anat. Rec.*, 1934, **60**, 43.

[8] Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 214.

[9] Wolfe, J. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 184.

[10] Barnes, B. O., Reagan, J. F., and Nelson, W. O., *J. Am. Med. Assn.*, 1933, **101**, 926.

[11] Nelson, W. O., and Overholser, M. D., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 150.

ture of the anterior lobe of the hypophysis and suppresses its sex-stimulating power and diabetogenic activity, it seemed worth while to investigate any possible influence it might have upon the other anterior pituitary effects, somatogenic, thyreotropic, and adrenotropic.

Litter-mate rats were divided into 3 groups. Group A, consisting of 3 males and 3 females, received daily injections of 9 rat units of theelin* in oil (0.03 cc.). Group B, comprising the same number of animals, was treated with injections of an equal quantity of Mazola oil. Group C, 2 males and 2 females, served as untreated controls. Injections were made daily from the 23d to the 90th day of life.

TABLE I.
Body Weight of Animals During Theelin Treatment, and of Controls.
Aver. Body Wt. in Gm.

| Age in days | 23 | 29 | 37 | 46 | 57 | 67 | 75 | 84 | 90 |
|---|---|---|---|---|---|---|---|---|---|
| Group A | 35 | 50 | 74 | 101 | 129 | 155 | 166 | 176 | 182 |
| ,, B | 34 | 45 | 71 | 100 | 133 | 162 | 176 | 188 | 193 |
| ,, C | 34 | 48 | 75 | 107 | 131 | 151 | 166 | 181 | 187 |

TABLE II.
Weights of Thyroids, Adrenals, and Gonads. No. of Animals Indicated in Parentheses. Both Gonads, Adrenals, and Thyroid Lobes Weighed Together.

| | Thyroid, aver. wt. in gm. | Adrenals, aver. wt. in gm. | Ovaries, aver. wt. in gm. | Testes, aver. wt. in gm. |
|---|---|---|---|---|
| Group A | 0.020 (6) | 0.052 (6) | 0.052 (3) | 2.719 (3) |
| ,, B | 0.020 (6) | 0.047 (6) | 0.062 (3) | 2.954 (3) |
| ,, C | 0.017 (4) | 0.041 (4) | 0.046 (2) | 2.711 (2) |

Results are shown in Tables I and II. No appreciable influence upon growth was noted, Groups A, B, and C averaging in weight respectively 35, 34, and 34 gm. at the outset, and 182, 193, and 187 gm. at the termination of the experiment. Nor was any essential difference found when the males and females of each group were considered separately. Likewise, no significant difference was observed in the weights of adrenals and thyroids of the treated animals as compared with those of the 2 control groups.

It is the common finding that the initial effect of theelin upon the gonads is an inhibition of growth and we have observed this regularly in other studies. That this effect is only temporary is indicated by this experiment, the testes and ovaries showing normal size after 67 days of treatment. The vaginae of group A were all open by the 27th day of life, those of groups B and C, from the 49th to the 59th day.

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*The theelin was kindly supplied by Dr. O. Kamm, Parke, Davis and Company.

The histological picture of the adrenals, thyroids, testes, and ovaries of the theelin-treated animals differed in no respect from those of the 2 control groups.

Our experiments indicate that theelin has no effect upon the somatogenic, thyreotropic, or adrenotropic activity of the anterior pituitary of rats.

## 8180 C

## Experimental Production of Glandular Cystic Hyperplasia of Endometrium with Estrogenic Substances of Parturient Urine.

JOHN W. SIMPSON AND JOHN C. BURCH.

*From the Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine, Nashville, Tenn.*

In a recent review of the important gynecological condition of glandular cystic hyperplasia of the endometrium, attention was called to the wide divergence of opinion concerning the amount of estrogenic substance necessary to produce it.[1] The enormous proliferation of the endometrium and the finding of increased amounts of an estrous inducing substance in the blood and urine of a certain percentage of these cases point to an excessive production of estrogenic hormones, as a result the disease is commonly referred to as an hyperestral one, or is said to exhibit hyperestrinism. Evans,[2] Novak,[3] Siebke,[4] Zondek,[5] Frank,[6] Kurzrok[7] and Fluhmann[8] all seem to favor this view. On the other hand the occurrence of the disease in sterile women, its close relation to amenorrhea and its occurrence near the menopause and after partial castration indicate that there is a decreased function of the ovary and a lowered ovarian secretion. Seitz[9] and Mazer and Goldstein[10] have taken this latter view.

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[1] Burch, John C., Phelps, Doris, and Wolfe, J. M., *Arch. Path.*, 1934, **17**, 799.

[2] Evans, H. M., *J. A. M. A.*, 1935, **104**, 464.

[3] Novak, Emil, *Surgery, Gynecology and Obstetrics*, 1935, **60**, 330.

[4] Siebke, Harold, *Zentralblatt f. Gyn.*, 1929, **53**, 2450.

[5] Zondek, Bernhard, *Acta Obstet. et Gyn. Scandinavica*, 1934, **13**, 309.

[6] Frank, Robert T., Goldberger, Morris A., Spielman, Frank, *J. A. M. A.*, 1934, **103**, 393.

[7] Kurzrok, Raphael, *Endocrin.*, 1932, **16**, 361.

[8] Fluhmann, C. F., *Surgery, Gynecology and Obstetrics*, 1931, **52**, 1051.

[9] Seitz, L., *Munchen Med. Wochr.*, 1930, **77**, 133.

[10] Mazer and Goldstein, W. B. Saunders, Philadelphia, 1932.

Due to the rapidly changing opinion as to the rôle of the estrins in the hypophyseal-ovarian complex, and their close relationship to glandular cystic hyperplasia of the endometrium, it seemed important to us to consider the question of the amount necessary to produce glandular cystic hyperplasia of the endometrium.

Leaving aside all questions of the accuracy of the biological methods employed in the assay of the estrin content of the human blood and urine, the difficulties encountered in obtaining a sufficiently large number of specimens from the same women in the various phases of the disordered menstrual cycle is so great that it seemed wiser to us to study the changes produced in the castrate guinea pig endometrium by known quantities of labor urine containing a high percentage of estrogenic substances and to measure these changes in terms of vaginal estrous. The guinea pig was selected as our experimental animal on account of its occasional exhibition of the spontaneous occurrence of the condition; the ease of its experimental production in the castrate by injections of estrogenic substance and the well marked estral changes in this species.

Thirty-four adult, castrated female guinea pigs were injected with varying amounts of the same batch of boiled labor urine. Sections of the endometrium were taken for microscopic study on the 21st or 28th day. Vaginal smears were taken daily and carefully checked. The results can be most easily considered by dividing the animals into 3 groups.

In the first of these were 5 animals, who received from .0075 cc. to .005 cc. per gram of body weight of the boiled labor urine containing the estrin. All showed well marked estrous smears and all had a well developed endometrial hyperplasia of the cystic glandular type. In the second group nine animals received .004 cc. to .005 cc. which was gradually reduced to .002 cc. during the course of the experiment. None of these ever showed any estral changes in the smears and hyperplasia was found in only 4. In the third group, 20 animals received amounts of .002 or less. The amount given each animal was not varied from day to day, but was kept at the same level. None came into estrous and all had hyperplasia.

The experiments show that the amounts of estrogenic substance necessary to produce glandular cystic hyperplasia in guinea pigs is less than that required for vaginal estrous.

8181 C

## Influence of Electrolytes Added to Growth Medium on Electrophoretic Potential of Escherichia Coli.*

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(Introduced by R. Adams Dutcher.)

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Aqueous suspensions of *Esch. coli* picked from 24-hour growths at 37.5°C. on Bacto-nutrient agar adjusted in pH from 6.2 to 7.9 gave slightly lesser (to 4%) cataphoretic velocities, as measured in Falk capillary cells[1] than did suspensions washed one to 3 times. Accordingly, we used a suspension produced by washing the growth from the medium, centrifuging once and resuspending the bacteria in distilled water, in a study of the effect of the addition of salts to the growth medium on the electrophoretic velocity of *Esch. coli*

TABLE I.

| Salt added | Concentration in medium | Migration velocity‡ Av. of 60-80 Readings, sec. | % change from control |
|---|---|---|---|
| NaCl | 0 (Control) | 8.06 | — |
| ’’ | 10–4 Molar | 8.04 | —0.25 |
| ’’ | 10–3 ’’ | 8.01 | —0.62 |
| ’’ | 10–2 ’’ | 7.92 | —1.74 |
| $CaCl_2$ | 0 (Control) | 9.18 | — |
| ’’ | 10–4 Molar | 8.63 | —5.99 |
| ’’ | 10–3 ’’ | 8.58 | —6.54 |
| ’’ | 10–2 ’’ | 8.47 | —7.73 |
| $AlCl_3$ | 0 (Control) | 8.08 | — |
| ’’ | 10–4 Molar | 8.05 | —0.37 |
| ’’ | 10–3 ’’ | 7.99 | —1.11 |
| ’’ | 5 x 10–3 ’’ | 8.03 | —0.62 |
| $Na_2SO_4$ | 0 (Control) | 8.04 | — |
| ’’ | 10–4 Molar | 8.46 | +5.22 |
| ’’ | 10–3 ’’ | 8.46 | +5.22 |
| ’’ | 10–2 ’’ | 8.44 | +4.98 |
| $Na_3PO_4$ | 0 (Control) | 7.98 | — |
| ’’ | 10–4 Molar | 8.03 | +0.63 |
| ’’ | 10–3 ’’ | 8.01 | +0.38 |
| ’’ | 10–2 ’’ | 8.00 | +0.25 |

‡ Migration velocity = Electroendosmotic velocity minus cataphoretic velocity, voltage and distance being constant.

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* Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, May 16, 1935, as Technical Paper No. 689.

† Master's thesis.

[1] Falk, I. S., Jensen, L. B., and Mills, J. H., *J. Bact.*, 1928, **15**, 421.

as measured with the Falk capillary cell. As Table I will show, the addition of the salts to the growth medium had little or no effect on the cataphoretic velocity which measures the zeta potential. In a future paper (Pedlow and Lisse) it will be shown that the zeta potential of the bacteria in aqueous suspension can be changed when salts are added to the growth medium in greater concentrations.

It was also shown that no change of the electrophoretic velocity of organisms (once washed) greater than 2.5% was produced by adjusting the initial pH of the medium to values over the range 6.8 ± 1.9, the pH after growth having a value 6.5 to 8.6, 5.7 to 8.6, and 6.1 to 8.6 for the upper half, lower half and the whole agar.

### 8182 C

## Amino Acids in Human Skin.*

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The outer layers of human skin were successively extracted in the cold with 95% alcohol, ethyl ether and chloroform. The material was then digested with commercial pepsin for 72 hours and for a similar length of time with commercial trypsin. The residual dry product contained 6.1% of ash and 14.2% of total nitrogen. Some of it was analyzed by means of the Van Slyke partition method. Cystine was determined according to Folin and Marenzi and tyrosine and tryptophane by the Folin and Ciocalteu procedure. The values for the basic amino acids (Van Slyke method) were recalculated and are shown in the table in terms of per cent in the residual skin. This was done in order that a comparison could be made with the results obtained by Wilkerson,[1] who recently reported on the chemical nature of human skin. Wilkerson used a modification of the Vickery and Leavenworth method for the basic amino acids, but the remainder of his data were obtained by the same procedures employed by the writer. The values shown in Table I are those obtained by the writer as well as those pub-

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* This material was made available through the courtesy of Professor Udo J. Wile of the Department of Dermatology and Syphilology, Medical School, University of Michigan, Ann Arbor.

[1] Wilkerson, V. A., *J. Biol. Chem.*, 1934, **107**, 377.

TABLE I.

| | Eckstein<br>% | Wilkerson[1]<br>% | Block[2]<br>% |
|---|---|---|---|
| Humin N | 2.02 | 2.11 | — |
| Amide N | 7.68 | 3.60 | — |
| Arginine | 5.91 | 10.01 | 6.00 |
| Lysine | 4.68 | 3.06 | 4.50 |
| Histidine | 0.64 | 0.59 | 0.82 |
| Cystine | 3.82 | 2.31 | 3.40 |
| Tyrosine | 3.42 | 5.70 | — |
| Tryptophane | 1.80 | 1.49 | — |

lished by Wilkerson. Those given for human nitrogen and amide nitrogen are expressed in terms of total nitrogen while the ones shown for the amino acids are given in terms of the residual tissue. It is clear from the tabulations that a considerable difference exists between the two sets of data. This may be due to the fact that whereas the author analyzed skin that had been digested with proteolytic enzymes, Wilkerson merely examined partially defatted skin. On the other hand, Block[2] who digested skin with pepsin found 6.0% of arginine, 4.5% of lysine, and 0.82% of histidine in his dry digested material. Block's results are in accordance with the writer's. Block also used a modification of the Vickery and Leavenworth method.

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8183 C

## Basic Amino Acids of Human Skin.*

RICHARD J. BLOCK.†

*From the Biochemical Laboratory of the Connecticut Agricultural Experiment Station and the Department of Physiological Chemistry, Yale University, New Haven.*

The older definition of keratin based on the physical properties and behavior towards the common proteolytic enzymes of the tissue proteins has been modified by Block and Vickery.[1] They define keratin as "a protein which is resistant to digestion by pepsin and trypsin, which is insoluble in dilute acids and alkalies, in water and in organic solvents, and which on acid hydrolysis, yields such quantities of histidine, lysine, and arginine, that the molecular ratios of these amino acids are respectively approximately as 1:4:12." However, proteins which do not yield approximately such quantities of the basic amino acids even though they fulfill the other criteria of the definition are not considered by Block[2] to be true keratins. Thus, for example, neurokeratin, which is insoluble in dilute acids and alkalies, not digested by pepsin and trypsin, etc., was considered *not* to be a true keratin because it yielded histidine, lysine, and arginine in the molecular ratios of 1:2:2.

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[2] Block, R. J., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1574.

* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and by the Carnegie Institution of Washington, D. C.

† The data in this paper are taken from the dissertation submitted by R. J. Block in partial fulfillment of the requirement for the degree of Doctor of Philosophy, Yale University, 1931.

[1] Block, R. J., and Vickery, H. B., *J. Biol. Chem.*, 1931, **93**, 113.

Human skin (cornified epithelium from the sole of the foot and scales from patients with exfoliative dermatoses of various kinds) was obtained through the kindness of Dr. H. S. Burr of New Haven, Dr. H. B. Lewis of Ann Arbor, and Dr. G. M. McKee of New York City. The washed skin was extracted with hot alcohol, ether, and digested with pepsin-hydrochloric acid. Fourteen gm. of the dried skin, after hydrolysis, yielded 6.0% of arginine, 0.82% of histidine, 4.3% of lysine, and 3.4% of cystine. The basic amino acids were isolated by the silver precipitation method[3] and cystine was determined colorimetrically.[4] These results agree remarkably well with those reported by Eckstein[5] who used the Van Slyke nitrogen distribution method.

The molecular ratios of histidine:lysine:arginine in the samples of skin analyzed by Eckstein and by ourselves are of the order of 1:6:7 and, therefore, the samples of tissue analyzed, although insoluble and indigestible, are not in strict conformance with the definition of true keratins. On the other hand, the sample of epidermal scales analyzed by Wilkerson[6] obtained from a patient suffering from dermatitis exfoliativa yielded histidine, lysine, and arginine in the molecular ratios of 1:6:15, characterizing this sample of tissue as a true keratin. So, it appears possible that the process of keratinization had been completed in the sample of skin studied by Wilkerson, but that these changes were still in progress in the tissues analyzed by Eckstein and by ourselves.‡

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[2] Block, R. J., *J. Biol. Chem.*, 1932, **94**, 647.

[3] Vickery, H. B., and Block, R. J., *J. Biol. Chem.*, 1931, **93**, 105.

[4] Folin, O., and Marenzi, A. D., *J. Biol. Chem.*, 1929, **83**, 103.

[5] Eckstein, H. C., (see accompanying paper).

[6] Wilkerson, V. A., *J. Biol. Chem.*, 1934, **107**, 377.

‡ I am indebted to Dr. H. B. Vickery for advice and for helpful comments during the course of this investigation.

## 8184 P

# Excretion of Follicle-Stimulating Hormone in Urine of Mental Patients in and Past Menopause.*

M. M. HARRIS, E. BRAND, L. E. HINSIE, AND R. J. BLOCK.

*From the Departments of Internal Medicine, Chemistry and Clinical Psychiatry, N. Y. State Psychiatric Institute and Hospital, New York City.*

In a previous communication[1] the daily urinary excretion of gonadal stimulating substances was investigated over prolonged periods of time in a group of 15 mental patients and 5 non-psychiatric subjects. Female patients, in the involutional period, were found to excrete large amounts of follicle-stimulating hormone (F.S.H.). However the high level of excretion was not maintained continuously and on some days no hormone appeared to be excreted.

In the present study† the excretion of F.S.H. was investigated in a group of 94 psychotic women in and past the menopause (age 43-108 years). There were 13 patients diagnosed as manic-depressive psychoses, 8 as involutional melancholia, 17 as psychoses with cerebral arteriosclerosis, 39 as paranoid dementia praecox and 17 patients in other diagnostic categories. Three or 4 non-consecutive 24-hour specimens of urine were collected from each patient. Creatinine determinations were carried out on the individual specimens to ascertain the completeness of the collections.

A hormone concentrate was prepared from each 24-hour specimen of urine by essentially the same method used in our previous study.[1] This concentrate was injected twice daily for 3 days into an immature female rat (21-22 days old) from our stock colony. On the fifth day the ovaries were removed, weighed and examined. The ovaries obtained from at least one of the test periods from each patient were sectioned. The patency of the vagina and the uterine reaction were noted.

F.S.H. produces an increase in the weight of the ovaries, which increase apparently depends upon the amount of hormone injected. Therefore, the amount of hormone present in a 24-hour specimen of urine was approximated by means of the ovarian weight of the experimental animal. The normal weight of a pair of ovaries from our 24- to 25-day-old rats is 12 to 16 mg.

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* Presented at the 91st Annual Meeting of the American Psychiatric Association, Washington, D. C., May, 1935. Aided in part by a grant from the Thomas W. Salmon Memorial Fund.

[1] Harris, M. M., Brand, E., and Hinsie, L. E., *Am. J. Psychiatry*, in press.

† We are indebted to Doctors Merriman and Stein of the Manhattan State Hospital and Doctors Blaisdell, Chandler, Munn and Klein of the Rockland State Hospital for making patients available to us for this study.

The findings, thus far, of this study, which is still in progress, suggest the following:

(1) Patients with manic depressive psychosis and involutional melancholia appear to excrete larger amounts of F.S.H. than those suffering from paranoid dementia praecox and psychoses with cerebral arteriosclerosis. The F.S.H. excreted over 24 hours by the patients in the first 2 groups produced rat ovaries weighing on the average 120 mg., while the hormone excreted by the other groups gave ovaries averaging 80 mg. The first 2 groups also seemed to show somewhat less variability in their daily hormone excretion.

(2) There appeared to be a diminished excretion of F.S.H. in the paranoid dementia praecox and cerebro-arteriosclerotic patients who were beyond the age of 60 years. It is interesting to note, however, that a colored patient with senile psychosis and of the verified age of 108 years excreted daily relatively large and constant amounts of F.S.H., the rat ovaries weighing on the average 120 mg. (Case No. 6).

(3) The largest excretion of F.S.H. per 24 hours (303 mg. ovaries) occurred in a patient (No. 5), age 58 years, with hypertension and psychosis with cerebral arteriosclerosis.

(4) Contrary to certain reports in the literature, hypertension was not necessarily associated with a high level of hormone excretion. In fact, some patients with low blood pressure excreted more hormone than some of the cases in the hypertensive group.

(5) A poor state of nutrition, which seldom occurred in our patients, did not necessarily result in a low F.S.H. excretion. However, 3 dysplastic and markedly obese patients were observed to excrete a very small amount of hormone.

### 8185 P

## Effect of Coronary Artery Occlusion on Dog's Heart with Total Coronary Sinus Ligation.*

LOUIS GROSS AND LESTER BLUM.

*From the Laboratories of the Mount Sinai Hospital, New York City.*

One of us (L.G.)[1] described 3 vascular mechanisms in the blood supply to the human heart which probably serve as compensatory means to ward off the results of coronary artery narrowing or occlusion. The first and most important of these is a gradual and consistent widening of anastomotic channels which occurs with increasing age. It was shown that many of these anastomotic channels are situated in the interventricular septum. The second compensatory mechanism is the age period development of rami telae adiposae, vessels which lie in the epicardial mantle and anastomose with the myocardial vascular ramifications on the one hand, and with periaortic and peripulmonic vessels on the other. The third mechanism is the existence of anastomoses between the myocardial and pericardial vascular ramifications and the extracardiac vessels (bronchial arteries, arteriae mammariae internae and diaphragmmatic vessels). These studies were subsequently confirmed and extended by Campbell,[2] Davis,[3] Robertson,[4] Hudson, Moritz and Wearn,[5] and by Gross and Kugel.[6]

Recently, augmentation of these extracardiac anastomoses was attempted by Moritz, Hudson and Orgain[7] by means of epicardiectomy (stripping of epicardium) with resulting pericardial adhesions. Using this procedure, Beck, Tichy and Moritz[8] observed that some dogs may recover after gradual coronary artery occlusion covering a period of 2 years and that no infarctions were produced in the hearts. Robertson[9] produced pericardial adhesions by tying off

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* Aided by grants from the Lucius N. Littauer and Walter W. Naumburg Funds.

[1] Gross, Louis, The Blood Supply to the Heart, New York, 1921, Paul B. Hoeber.

[2] Campbell, J. S., *Quart. J. Med.*, 1929, **22**, 247.

[3] Davis, D. J., *Arch. Path. and Lab. Med.*, 1927, **4**, 937.

[4] Robertson, H. F., *Am. J. Path.*, 1930, **6**, 209.

[5] Hudson, C. L., Moritz, A. R., and Wearn, J. T., *J. Exp. Med.*, 1932, **56**, 919.

[6] Gross, Louis, and Kugel, M. A., *Am. Heart J.*, 1933, **9**, 165.

[7] Moritz, A. R., Hudson, C. L., and Orgain, E. S., *J. Exp. Med.*, 1932, **56**, 927.

[8] Beck, C. S., Tichy, V. L., and Moritz, A. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 759.

[9] Robertson, H. F., *Arch. Path.*, 1934, **18**, 575.

coronary veins in stages. In many cases such dogs tolerated coronary occlusion, also produced in stages. If the adhesions were separated, however, the dogs died with fresh infarcts. Robertson believed that the progressive adhesions supplied nutrition to the heart.

Apart from the fact that these techniques involve extensive operative procedures, the question arises as to whether the results obtained were due to the added vascular supply by way of the pericardium or to the compensatory vascular dilatation within the myocardium made possible by the slow occlusion of the coronary arteries.

The experiments here described indicate a method which apparently produces a striking increase in the blood supply to the heart by means of a simple operative procedure. For certain theoretical reasons it was believed that tying off the coronary sinus would hasten the physiological age period widening of anastomotic channels in the myocardium such as normally occurs in the human heart. With this in view, the following experiments were undertaken.

Under Pernoston or Nembutal anesthesia, the left anterior descending coronary branch was divided between ligatures in a number of dogs approximately 2 cm. below the aortic ostium of the left coronary artery. Of 20 dogs which survived this procedure for longer than one hour, 19 presented a large vascular filling defect (incomplete filling of the coronary tree) as determined by subsequent injection of the hearts with a barium sulphate suspension in gelatine followed by roentgenography.[1] In 16 of these dogs which were sacrificed at intervals varying from 18 hours to 12 weeks, an infarct, usually measuring 5x5.5 cm., was observed on the surface of the left ventricle. This infarct generally involved a large part of the interventricular septum. One dog showed a small infarct measuring 1x1 cm. The 3 remaining dogs died or were killed within a few hours after coronary ligation, a period of time too short to produce visible infarction. Apparently, therefore, this procedure produces infarction practically invariably in the dog's heart. This becomes grossly visible 18 hours after the occlusion.

In another group of 41 dogs coronary sinus ligation was performed. This was followed almost immediately by considerable dilatation and widening of existing vascular channels on the surface of the heart. A number of these animals were sacrificed for anatomical studies. Barium sulphate gelatine injection of the coronary tree in these hearts showed a conspicuous increase in the extent of the vascular bed. In 10 surviving animals the left anterior descending coronary branch was subsequently occluded in the manner described above. Eight of these animals died or were sacrificed in from 18 hours to 4 weeks after the arterial occlusion. The remaining 2 dogs died in less than 18 hours. These showed neither vascular filling defect (as determined by the injection technique) nor visible infarction. Of the 8 animals which died or were sacrificed 18 hours to 4 weeks after the arterial occlusion, 7 showed no vascular filling defect and 5 showed no infarction. The eighth animal showed a small vascular filling defect and infarction; another of the 8 animals showed a small infarct and one showed a myocardial hemorrhage. Apparently, therefore, in the majority of instances preliminary coronary sinus ligation prevents the appearance of infarction following acute occlusion of the left anterior descending coronary branch, and materially reduces the size of the infarct in others.

The operative mortality (within 48 hours after coronary sinus ligation) was 44% during our earlier experiences. This dropped to 20% in the last 10 dogs. Subsequent reports will deal with the technique employed and with certain characteristic electrocardiographic and other changes which follow these operative procedures.

It is hoped to apply this procedure to the human heart in order to increase the vascular tree.

## 8186 C

## Birth Weight Criterion of Dwarfism in the Rabbit.

PAUL D. ROSAHN AND HARRY S. N. GREENE.

*From the Laboratories of the Rockefeller Institute for Medical Research.*

The procedure practiced in this laboratory with reference to birth weight determinations is as follows: Each morning rabbits born during the previous 24-hour period are identified by color markings or toe amputations and weighed to the nearest gram on a balance calibrated in one gram intervals. The average elapsed time from birth to weighing is 12 hours, the mid-point of the 24-hour interval between determinations. It is apparent that this routine introduces an error which is dependent on the amount of nourishment which each individual in the litter has obtained between birth and the determination, since the female rabbit suckles her young immediately following parturition. This error will vary with the length of the interval between birth and the weight determination, and also with the number in the litter and the relative strength or weakness of each animal in it. The weaklings are frequently overpowered by their stronger sibs in the struggle for nourishment, and, moreover, the doe sometimes disregards her weaker offspring and refuses to nurse them. As a result, the weights recorded may deviate from the actual birth weights in a positive or negative direction, according to the relative strength or weakness of the animals comprising the litter.

The comparatively frequent birth of extremely small individuals in our colony has emphasized the desirability of ascertaining to what extent adverse conditions effective after birth could account for these dwarf-like animals, and also to establish quantitative criteria for the diagnosis of dwarfism in the rabbit. For this purpose an analysis was made of the variability in the birth weights of 565 individuals in 103 litters selected at random from the mating records of normal hybrid animals during 1933-1935. In all cases routine birth weight determinations were employed. The weight of each animal was expressed in terms of a percentage of the weight of the heaviest litter-mate. This procedure eliminates the variation due to differences in the absolute weights of animals of different litters since all observations are given a relative value. It is obvious also that the use of this method presupposes the presence of at least 2 animals in each litter. The resulting percentage values were grouped in classes in descending order of magnitude, and the frequencies in each class are shown in Table I and Fig. 1. It is seen that the frequencies describe a fairly orderly decrease from the highest number in the 100-90.1% class to the lowest in the 40.0-30.1% class. Birth weights exceeding 50.0% of the weight of the heaviest littermate were noted in 561 or 99.3% of the 565 animals. A review of the ancestry of the 4 animals whose weights fell below the 50.1% class, in each case showed related litters con-

TABLE I.

Frequency Distribution of Animals Comprising Normal and Dwarf Litters. Weight of Each Individual Expressed as a Percentage of the Weight of the Heaviest Littermate.

| Group | 100-90.1% | 90.0-80.1% | 80.0-70.1% | 70.0-60.1% | 60.0-50.1% | 50.0-40.1% | 40.0-30.1% | 30.0-20.1% | Total |
|---|---|---|---|---|---|---|---|---|---|
| Normal | 283 | 154 | 87 | 27 | 10 | 3 | 1 | 0 | 565 |
| Dwarf | 189 | 98 | 42 | 12 | 7 | 42 | 81 | 17 | 488 |
| Total | 472 | 252 | 129 | 39 | 17 | 45 | 82 | 17 | 1053 |

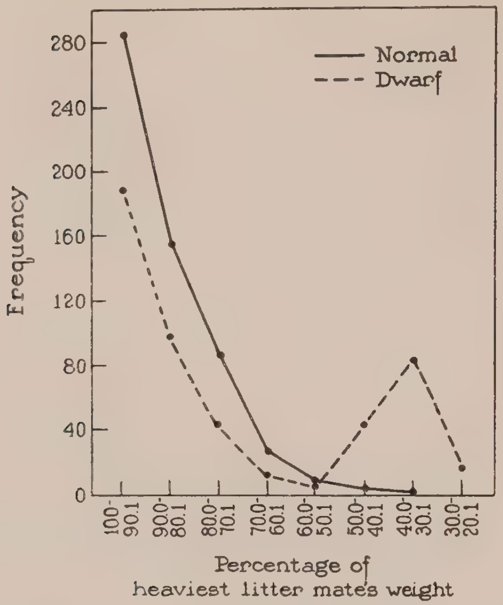

FIG. 1.

Frequency distribution of animals comprising normal and dwarf litters. Weight of each individual expressed as a percentage of the weight of the heaviest littermate.

taining similar small animals, and on this basis these 4 should probably be excluded from the normal group. The results of this analysis indicate that any adverse nutritive conditions which might be effective between birth and weighing are not responsible for animals whose weights are less than 50.1% of the weight of their heaviest littermate.

In connection with studies on a lethal dwarf mutation in the rabbit,[1] a similar analysis was made of the variability in the birth weights of individuals in litters containing unusually small, delicately formed living but nonviable young derived from known transmitters of the abnormality. The results are shown in Table I and Fig. 1. Here again, the frequencies in the different classes describe an orderly decrease from the 100-90.1% to the 60.0-50.1% class. The incidence in the succeeding groups differs radically from the previously described normal findings, as shown by the sharp rise to a high point in the 40.0-30.1% class followed by a precipitous fall in the 30.0-20.1% grouping. Of the 488 young, 348 or 71.3% had birth weights amounting to more than 50.0%

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[1] Greene, H. S. N., Hu, C. K., and Brown, W. H., *Science*, 1934, **79**, 487.

of that of their heaviest littermate, and this is significantly fewer than the comparative figure for the normal group ($n = 1$, $\chi^2 = 172.3$). Of great interest is the fact that the frequencies for the normal and dwarf litters describe significantly identical trends from the 100-90.1% to the 60.0-50.1% class ($n = 4$, $\chi^2 = 6.0$), indicating that the factors influencing variability between these extremes are operative with equal effectiveness on both groups.

*Conclusions.* When the birth weight of a rabbit as determined by the routine procedure described is less than 50.1% of the weight of his heaviest littermate, a diagnosis of dwarfism is warranted. The birth weights of unusually small, nonviable animals derived from known transmitters of the abnormality fall below the 50.1% class, and these animals are, therefore, genetically true dwarfs in the sense that their abnormally low weights cannot be ascribed to environmental and nutritive factors operating in the interval between birth and the weight determination.

## 8187 C

## Effect of Various Corticoadrenal Extracts on Diphtheria Toxin In Vivo and In Vitro.*

RAYMUND L. ZWEMER AND CLAUS W. JUNGEBLUT.

*From the Departments of Anatomy and Bacteriology, College of Physicians and Surgeons, Columbia University.*

This paper deals with the ability of various adrenal cortex extracts to raise the resistance of normal guinea pigs to injections of diphtheria toxin, and to inactivate the toxin *in vitro*. It is part of a research program[1] directed toward an understanding of the mechanism of natural resistance, and is an attempt to determine whether the adrenal cortex resistance factor can be isolated by the same chemical procedures used in extracting the vital hormone.

The 5 methods of corticoadrenal lipoid fractionation used are very briefly indicated on an accompanying composite chart (Table I). They have been studied by one of us (R.L.Z.) for a number of years and most of the essential principles have been reported by

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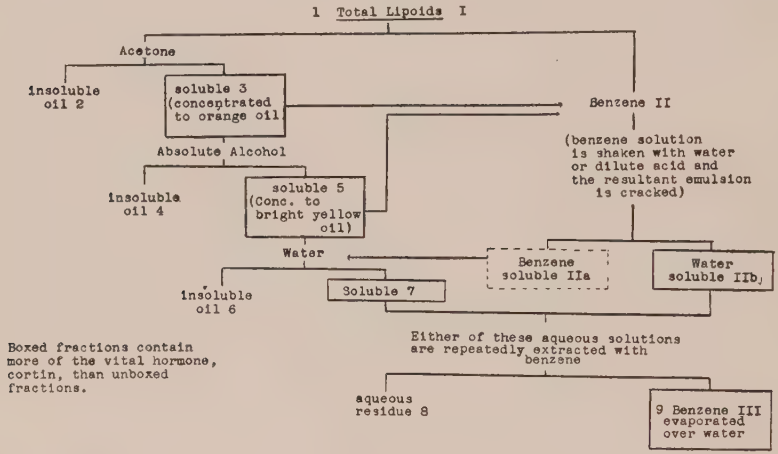
* Part of the expenses of this paper were defrayed by a grant from the Rockefeller Foundation.

[1] Jungeblut, C. W., and Zwemer, R. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1229.

TABLE I.

ADRENAL EXTRACT FRACTIONATION.†

Five procedures are shown, the essential principles of which have been used by a number of authors[2-8] in preparing corticoadrenal extracts. The finely ground fresh adrenal glands are simultaneously extracted with benzene (80°-82°) and dehydrated with anhydrous sodium sulphate. The benzene solution is then concentrated to a thick oil. Details concerning the various steps are to be reported elsewhere.


1 Total Lipoids I
Acetone
insoluble oil 2
soluble 3 (concentrated to orange oil)
Benzene II
(benzene solution is shaken with water or dilute acid and the resultant emulsion is cracked)
Absolute Alcohol
insoluble oil 4
soluble 5 (Conc. to bright yellow oil)
Water
Benzene soluble IIa
Water soluble IIb
insoluble oil 6
Soluble 7
Either of these aqueous solutions are repeatedly extracted with benzene
Boxed fractions contain more of the vital hormone, cortin, than unboxed fractions.
aqueous residue 8
9 Benzene III evaporated over water

workers on adrenal cortex extracts.[2-8] In addition to our own preparations, we used Eschatin (Parke, Davis & Co.) and 2 batches of corticoadrenal hormone kindly given us by Dr. Pfiffner of the Department of Biochemistry. One of his extracts had 100 dog units[9] and the other 40 dog units per cc.

The diphtheria toxin employed was from one batch of stabilized filtrate containing from 400-450 m.l.d. per cc., so that 1 cc. of a 1/200 dilution included at least 2 minimal lethal doses.

Over 150 guinea pigs were used as indicated in the tables and in the description of our results. Since many of the control animals accompanied the individual experiments with both corticoadrenal extracts and vitamin C, some of them were listed individually in the previous paper of this series.[1] In the present paper they are given in the first lines of Tables II, III and IV.

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† Many of these fractions were prepared in the Department of Anatomy by Messrs. L. Schwartz and Wm. Parsons during their work on another problem.

[2] Hartman, F. A., and Brownell, K. A., *Am. J. Physiol.*, 1930, **95**, 670.

[3] Swingle, W. W., and Pfiffner, J. J., *Am. J. Physiol.*, 1931, **96**, 165.

[4] Zwemer, R. L., Agate, F. J., Jr., and Schroeder, H. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 721.

[5] Kutz, R. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 91.

[6] Grollman, A., and Firor, W. M., *J. Biol. Chem.*, 1933, **100**, 429.

[7] Pfiffner, J. J., Vars, H. M., and Taylor, A. R., *J. Biol. Chem.*, 1934, **106**, 625.

[8] Pfiffner, J. J., and Vars, H. M., *J. Biol. Chem.*, 1934, **106**, 645.

[9] Harrop, G. H., Pfiffner, J. J., Weinstein, A., and Swingle, W. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 449.

*Intracutaneous tests* with 1/50, 1/100, 1/200 and 1/500 m.l.d. of diphtheria toxin were made on 46 light colored, 350 gm. guinea pigs. Certain fractions of adrenal cortex extract, when given in various

### TABLE II.

0 = negative
1 = very slight red (1 x 1 cm.)
2 = slight red (1½ x 1½ cm.)
3 = red (2 x 2 cm.)
4 = moderate necrosis
5 = heavy necrosis
ø = faded

Effect of Single Injections of Adrenal Cortex Extracts on Reaction of Normal Guinea Pigs to Small Intracutaneous Doses of Diphtheria Toxin.
(Corticoadrenal extracts were given 24 hours before toxin injections.)

| Guinea pig No. | Extract fraction | Amount given cc. | Reaction after 24 hours | | | | Reaction after 72 hours | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1/50 MLD | 1/100 MLD | 1/200 MLD | 1/500 MLD | 1/50 MLD | 1/100 MLD | 1/200 MLD | 1/500 MLD |
| Control average (16 animals) | | | 3 | 3 | 2 | 2 | 5 | 4 | 3 | 2 |
| 1 | 1 | 1 | 2 | 2 | 2 | 2 | 5 | 5 | 4 | 2 |
| 2 | 3 | 2 | 0 | 0 | 0 | 0 | 5 | 4 | 2 | 2 |
| 3 | 3 | 2 | 0 | 0 | 0 | 0 | 5 | 3 | 2 | 1 |
| 4 | 3 | 2 | 0 | 0 | 0 | 0 | 5 | 5 | 2 | 2 |
| 5 | 3 | 2 | 1 | 1 | 1 | 1 | 5 | 4 | 1 | 1 |
| 6 | 3 | 2 | 1 | 1 | 1 | 1 | 5 | 4 | 2 | 1 |
| 7 | 3 | 2 | 2 | 2 | 1 | 0 | 5 | 5 | 1 | 0 |
| 8 | 3 | 1 | 1 | 0 | 0 | 0 | 5 | 5 | 4 | 2 |
| 9 | 3 | 1 | 1 | 1 | 1 | 1 | 4 | 3 | 2 | ø |
| 10 | 3 | 1 | 2 | 2 | 2 | 1 | 4 | 3 | 2 | 2 |
| 11 | 3 | 1 | 3 | 3 | 3 | 2 | 5 | 4 | 4 | 2 |
| 12 | 5 | 1 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 |
| 13 | 5 | 1 | 0 | 0 | 0 | 0 | 4 | 4 | 4 | 2 |
| 14 | 5 | 1 | 3 | 2 | 2 | 2 | 4 | 2 | 2 | 2 |
| 15 | 5 | 0.5 | 1 | 1 | 1 | 0 | ø | ø | ø | 0 |
| 16 | 5 | 0.5 | 3 | 2 | 2 | 2 | 4 | 4 | 3 | 2 |
| 17 | 7 | 3 | 1 | 1 | 0 | 0 | 4 | 4 | 2 | 2 |
| 18 | 7 | 2 | 2 | 2 | 1 | 1 | 3 | 3 | 1 | ø |
| 19 | 7 | 1 | 2 | 1 | 1 | 1 | 4 | 4 | 3 | 2 |
| 20 | 9 | 0.5 | 1 | 1 | 1 | 0 | 4 | 3 | 3 | 2 |
| 21 | 9 | 0.25 | 2 | 2 | 1 | 0 | 5 | 4 | 3 | 2 |
| 22 | IIa | 2 | 2 | 2 | 2 | 1 | 5 | 5 | 4 | 1 |
| 23 | IIa | 1 | 3 | 3 | 2 | 2 | 5 | 4 | 2 | 2 |
| 24 | IIa | 0.5 | 2 | 1 | 1 | 0 | 5 | 4 | 4 | 2 |
| 25 | 2 | 1 | 3 | 3 | 2 | 2 | 5 | 3 | 2 | 1 |
| 26 | 4 | 2 | 2 | 1 | 1 | 1 | 4 | 3 | 2 | ø |
| 27 | 4 | 1 | 2 | 2 | 1 | 0 | 5 | 4 | 4 | 3 |
| 28 | 4 | 1 | 3 | 3 | 2 | 2 | 5 | 4 | 4 | 2 |
| 29 | 6 | 1 | 3 | 3 | 3 | 3 | 5 | 5 | 5 | 3 |
| 30 | 8 | 1 | 3 | 2 | 2 | 2 | 5 | 4 | 3 | 2 |

Compare fractions 2 and 3; 4 and 5; 6 and 7; 8 and 9 for relative activity.

doses (depending upon their toxicity) 24 hours before toxin, diminished or prevented the characteristic skin reactions (Table II). Results with other fractions did not differ greatly from those observed in the 16 controls.

By comparing the extract fraction numbers in Tables I and II it can be seen that the toxin-inhibitory fractions are those possessing relatively larger amounts of cortin, the vital hormone of the adrenal cortex. They were administered both as oils and also as emulsions in water. The lack of protection shown by non-hormonal phospholipin or sterol fractions argues against neutralization of toxin by adsorption onto the surface of lipoid droplets.

Another control substance used, *i. e.,* olive oil, proved to have some anti-diphtheritic properties. This observation confirmed some recent work[10, 11] and seemed to favor the possibility of lipoid adsorption. However, naturally occurring, neutral fatty oils have a small amount of other lipoids mixed with them. Upon extraction with cold absolute ethyl alcohol these proved to have enhanced anti-diphtheritic properties, whereas, the large alcohol-insoluble fraction of olive oil was no longer effective. This is in harmony with a recent report[12] which states that crude lipoids on purification lose their power of assisting tissue immune reactions. Our observation may help to explain the variable results obtained when toxins are inactivated by admixture with lipoid emulsions.

The cell protective action of our aqueous corticoadrenal hormone preparations is supported by 6 experiments with Pfiffner's extract and Eschatin. These were apparently more effective when given 4 to 8 hours before the injection of the toxin. Since the time necessary for absorption into the body differs among the various oily and aqueous fractions used, it will obviously require a large number of tests with graded doses and time intervals for an exact comparison of relative potencies.

The negative skin reaction does not seem to be due to the possible presence of vitamin C in our extracts, since it requires 50 mg. to 100 mg. of cevitamic acid per day for 6 days[1] to bring about a comparable effect. However, the possibility of a synergistic action between cortin and Vitamin C has to be kept in mind.

*In vitro tests.* Sixty-three normal 250 gm. guinea pigs were injected subcutaneously with 2 m.l.d. of diphtheria toxin. The 24 control animals died within 72 hours. The 39 test animals received

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[10] Walsh, V. G., and Frazer, A. C., *Brit. Med. J.*, 1934, **1**, 424, 557.

[11] Myers, G. N., *Brit. Med. J.*, 1934, **1**, 945.

[12] Hanger, F. M., *J. Clin. Invest.*, 1934, **13**, 692.

1 cc. of toxin (2 m.l.d.) which had been mixed with 1 cc. of an adrenal extract fraction. The mixture had been allowed to stand at room temperature for ½ hour. More than 35% of the 28 guinea pigs receiving mixtures which contained cortin survived the test (Table III). Another 25% showed prolongation over the control period. Very potent hormone gave a better average than that shown, but we have included all animals used to determine the least amount that would inactivate 2 m.l.d. of toxin. This was found to be roughly equivalent to 1 gm. of beef adrenal. Eleven animals receiving non-hormonal fractions did not differ greatly from the controls. These results confirm previous *in vitro* studies[13] and favor the view that cell protective activity accompanies cortin in its chemical isolation.

TABLE III.

Effect of 2 MLD of Diphtheria Toxin on 63 Normal 250 gm. Guinea Pigs, Alone and After Mixing with 1 cc. of Various Hormonal and Non-hormonal Fractions of Corticoadrenal Extracts.

| No. of guinea pigs | Type of treatment | Died within 72 hr. % | Lived 4-9 days % | Survived % |
|---|---|---|---|---|
| 24 | Controls | 100 | 0 | 0 |
| 11 | Non-hormonal fractions (2, 4, 6, 8, IIa) | 82 | 9 | 9 |
| 18 | A. C. hormone fractions (1, 3, 5, 7, 9) | 45 | 22 | 33 |
| 10 | Pfiffner A. C. hormone extract | 30 | 30 | 40 |

These results have been condensed for clarity in presentation. Some of our fractions and Pfiffner's extract were found to be effective when the amount used was the equivalent of 1 gm. of beef adrenal or 2.5 dog units.

*Titration against 2 m.l.d. in vivo.* Forty-five guinea pigs weighing 250 gm. were inoculated subcutaneously with 2 m.l.d. of diphtheria toxin, and corticoadrenal extract was injected intramuscularly (Table IV). From 100 to 200 times as many gram equivalents seem to be necessary in order to obtain survival of the animal under such conditions than is required for inactivation of the toxin

TABLE IV.

Estimation of Beef Adrenal Gram Equivalent in Active Corticoadrenal Extracts Necessary to Counteract *in Vivo* the Effect of 2 MLD of Diphtheria Toxin on Normal 250 gm. Guinea Pigs.

| No. of guinea pigs | Treatment | Died within 72 hr. % | Lived 4-9 days % | Survived % |
|---|---|---|---|---|
| 24 | Controls‡ | 100 | 0 | 0 |
| 29 | A. C. extract 1 to 9 gm. | 93 | 7 | 0 |
| 10 | A. C. " 10 " 99 " | 80 | 20 | 0 |
| 6 | A. C. " 100 " 200 " | 50 | 0 | 50 |

‡ Same controls as in Table III.

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[13] Jungeblut, C. W., Meyer, K., and Engle, E. T., *J. Immunol.*, 1934, **27**, 43.

by direct contact with the hormone. Even with the most concentrated preparations only 50% of the animals survived. Weaker preparations sometimes delayed death for several days but in most cases were without effect.

These experiments indicate that extraction methods yielding the resistance promoting factor of the adrenal cortex parallel those used for the isolation of the vital hormone. Both end products are similar in certain of their chemical and physical properties. It would not be wise to speculate as to their identity, since even very potent adrenal cortex extracts contain several chemical entities.[14]

We do not believe that our active fractions contain a specific antitoxic substance, but rather a hormone which perhaps acts in a catalytic manner to decrease the permeability of cells to toxin. Best results are obtained when the maximal amount of hormone is in or available to the cells at the time of greatest demand. Coca, Russell and Baughman[15] felt that the natural resistance of the rat to diphtheria toxin was due to a property of its cells which prevented toxin from entering or becoming attached to them. This property diminishes after adrenalectomy.

Our work shows that injections of adrenal cortex extract give normal guinea pigs an increased cell resistance to toxin for one or 2 days. By the third day the toxin generally produces the characteristic necrosis. In some cases the potency of the adrenal cortex fraction was evidently so high that destruction of cells by toxin was negligible. Meanwhile other body mechanisms had probably either neutralized or otherwise disposed of the toxin. It must be noted that in the present experiments only single injections of extract were given. In counteracting the effects of other toxins[16] it was found that repeated daily doses were more effective.

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[14] Wintersteiner, O., and Pfiffner, J. J., *J. Biol. Chem.*, 1935, **109**, C.

[15] Coca, A. F., Russell, E. F., and Baughman, W. H., *J. Immunol.*, 1921, **6**, 387.

[16] Zwemer, R. L., and Spence, M. J., in preparation.

## 8188 C

# Gonadotropic Substance from Teratoma of the Testis.

S. C. FREED AND A. COPPOCK.* (Introduced by Samuel Soskin.)

*From the Department of Metabolism and Endocrinology, and the Department of Pathology, Michael Reese Hospital.*

The demonstration of an increased excretion of gonadotropic hormone in the urine of patients with teratoma testis was made by Zondek.[1] He found that this hormone stimulated chiefly the follicles in the ovaries of rats and mice, although slight luteinization did occur at high dosages. He, therefore, concluded that it was identical with "Prolan A" in contradistinction to "Prolan B", the luteinizing hormone of pregnancy urine. Ferguson[2] recently reported 117 cases of teratoma testis with positive Ascheim-Zondek tests due to "Prolan A". On the other hand, Fluhman[3] and Hamburger[4] both found that in their cases of teratoma testis the hormone was similar to that occurring in pregnancy urine. Fluhman substantiated this conclusion on evidence of its ineffectiveness in hypophysectomized rats and on the results of his 5-10-day test.

Evans'[5] findings on a case of teratoma testis resembled those obtained with pregnancy urine in that his tests showed synergism with a pituitary extract. His material, however, resembled pituitary hormone in its *unlimited* effect on ovarian growth, confined to follicular stimulation. In a case studied by Main and Leonard[6] the pure follicular response was also obtained, but the hormone resembled that of pregnancy urine in its *limited* effect on ovarian weight with unlimited dosage.

We have had occasion to examine the urine of a case of teratoma testis with metastases. The presence of chorionepitheliomatous tissue was not mentioned in the biopsy report. The Ascheim-Zondek test assayed 10,000 rat units per liter. The ovaries of animals autopsied 96 hours after starting injections showed an apparent "Prolan A" reaction. The ovaries were white with several large clear follicles presenting themselves. On the microscopic examination of these ovaries, however, we were surprised to find only a slight difference from those treated with pregnancy urine, a "Prolan B" reaction. The structures which upon gross examination appeared to be follicles were, in reality, lutein cysts. There were also medium sized corpora lutea and thecal luteinization about the smaller follicles. We were impressed also with the tendency of our teratoma urine to form even more numerous lutein cysts than are found after the administration of pregnancy urine. A closer examination disclosed that the corpora were practically avascular, the cells were tightly packed with very few intervening capillaries, in contrast with the corpora formed by pregnancy urine where almost every cell is in contact with a capillary. This poor blood supply explained the white appearance of the ovary and the lack of the pink or reddish corpora one finds on gross examination of the ovaries after pregnancy urine injections in rats.

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* We wish to thank Dr. Samuel Soskin for aid and direction.

[1] Zondek, B., Die Hormone des ovariums und des Hypophysenvorderlappens, Springer, Berlin, 1931.

[2] Ferguson, R. S., *J. A. M. A.*, 1933, **101**, 1933.

[3] Fluhman, C. F., *Am. J. Obs. and Gyn.*, 1934, **28**, 668.

[4] Hamburger, C., Studies on Gonadotropic Hormones, Copenhagen, 1933, Levin and Munksgaard.

[5] Evans, H. M., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 21.

[6] Main, R. J., and Leonard, S. L., *Endo.*, 1934, **18**, 629.

When this same urine was tested on guinea pigs, a massive thecal luteinization resulted, which was similar in all respects to that obtained with pregnancy urine.[7, 8]

In addition to its modified luteinizing effect in rats, our material resembled pregnancy urine in that it gave a limited ovarian response with increasing dosage, 150 units having no greater effect than 30 units. Furthermore, with the simultaneous injection of a pituitary extract rich in the synergist, we were able to demonstrate a marked augmentation in ovarian weight.

The urine from our case differed from pregnancy urine in that the uteri of the test rats were pale, heavy-walled with small lumina, in contrast to the hyperemic, fluid-distended uteri of rats injected with pregnancy urine.

*Summary.* The investigation of the urine obtained from a case of teratoma testis has yielded results similar to those obtained with pregnancy urine, except for the avascular corpora lutea, more numerous lutein cysts in the ovary, and a distinctly different uterine appearance. These differences from the typical pregnancy urine effect produce a gross appearance which may be mistaken for a "Prolan A" reaction. Consistent recourse to histologic examination may reveal more cases similar to our own, than is indicated by a perusal of the literature.

Granting the above possibility of error, it still seems necessary to explain the divergence of the results of various workers on the basis

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[7] Loeb, L., *Endo.*, 1932, **16**, 129.

[8] King, A. G., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1182.

of the occurrence of more than one kind of hormone elaborated by the teratoma testis.

## 8189 P

## Effects on Vascular-Renal System of Posterior Pituitary Extract Administered During Pregnancy.

WILLIAM J. DIECKMANN AND HERBERT L. MICHEL.

*From the Department of Obstetrics and Gynecology, the University of Chicago, and the Chicago Lying-in Hospital.*

The parenteral or intranasal administration of extracts of the posterior lobe and pars intermedia of the pituitary body produces a decrease in the volume of the urine, an increase in the concentration of the urinary chloride, and a slight, if any, rise in the blood pressure.[1] The duration of these changes varies with the amount of the extract, but the magnitude of the response seems to bear no relation to the size of the dose.

Kamm and his coworkers[2, 3] have obtained two fractions from an extract of the posterior lobe of the pituitary gland—pitressin and pitocin. Pitressin contains the pressor and antidiuretic components, and its action is similar to the whole extract. Pitocin contains the oxytocic principle.

Shortly after the isolation of pitressin and pitocin, Ward, Lyon and Bemis[4] compared the action of these substances with pituitrin on pregnant women. They stated that pitressin and pituitrin produced an increase in the systolic blood pressure of 17 and 10 mm., respectively, which lasted for fifteen minutes. Pitocin caused an increase of only 5 mm., returning to normal in 5 minutes. Unfortunately, no attention was devoted to the water balance and the study of the blood pressure changes was inadequate.

Chipman,[5] Dieckmann, and undoubtedly other obstetricians have stated that the use of pituitrin to induce labor in pre-eclamptic patients has occasionally seemed to initiate the onset of convulsions. Convulsions and coma, of course, occur most frequently during labor, and the irritating effect of the uterine contractions may have produced the fits rather than the pressor and antidiuretic parts of pituitrin. An oliguria or anuria is one of the dominant signs of eclampsia and pre-eclampsia. We have noted that the oliguria is even more marked in severe and acute toxemic patients during labor and believe this disturbance in water balance might be the cause of the high incidence of intrapartum eclampsia.

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[1] Stehle, R., *Am. J. Physiol.*, 1927, **79**, 289.

[2] Kamm, O., Aldrich, T., Grote, I., Rowe, L., and Bugbee, E., *J. Am. Chem. Soc.*, 1928, **50**, 573.

[3] Bugbee, E., and Simond, A., *Am. J. Physiol.*, 1928, **86**, 171.

[4] Ward, G., Lyon, E., and Bemis, G., *Am. J. Obst. and Gynec.*, 1928, **16**, 655.

[5] Chipman, cited by Ward *et al.*

*Method of Study.* Retention catheters were inserted into the bladders of pregnant, parturient and puerperal women, and the urine collected hourly. Water in amounts of 150 to 250 cc. was given each hour. After a satisfactory volume of urine had been obtained, one-half to two minims of pituitrin were injected subcutaneously in the pregnant patients, and intravenously in those in the puerperium. Illustrative data are given in Table I.

TABLE I.

| Hourly Urine Output Before Pituitary Extract | | Hourly Urine Output After Pituitary Extract | | | | Rise in Systolic Blood Pressure |
|---|---|---|---|---|---|---|
| 2 | 1 | 1 | 2 | 3 | 4 | |
| | | Normal Pregnancy | | | | |
| 165 | 120 | 30 | 30 | | | 12 |
| 219 | 210 | 110 | 70 | | | 16 |
| 204 | 300 | 50 | 26 | 39 | 304 | 20 |
| 125 | 190 | 75 | 55 | | | 20 |
| 54 | 65 | 10 | 10 | 10 | 0 | 17 |
| | | Toxemia of Pregnancy | | | | |
| 30 | 16 | 10 | 10 | 75 | | 60 |
| 240 | 155 | 45 | 55 | | | 75 |
| 240 | 240 | 100 | 90 | | | 60 |
| 75 | 80 | 25 | 5 | 0 | 0 | 60 |
| 200 | 195 | 3 | 30 | 0 | 500 | 48 |
| 60 | 150 | 25 | 30 | 20 | 10 | 50 |
| 17 | 18 | 0 | 0 | 0 | 10 | 80 |

Ten normal pregnant and 21 toxemic patients have been studied to date. In the normal pregnant patients there is a very definite decrease in the volume of the urine and an increase in the chloride concentration, as is reported for non-pregnant individuals. In the toxemic patients, especially those diagnosed as pre-eclamptics, there is a very abrupt and marked increase in the systolic, as well as the diastolic, blood pressure, and a more marked decrease in the volume of urine. In one patient the oliguria was so marked that an acute pulmonary edema resulted. In another the pressor response was marked and may have been the cause of a partial premature separation of the placenta.

*Summary.* The volume of urine, providing the fluid intake is adequate, is not diminished during labor in normal pregnant women.

It is, however, decreased in pre-eclampsia or eclampsia, and the forcing of fluids may produce undesirable systemic symptoms and signs.

The parenteral injection of extracts of the posterior lobe of the pituitary gland produced the following changes: 1. A decrease in the volume of urine, an increase in the chloride concentration, and an average rise in the systolic blood pressure of 15 mm. Hg. in normal pregnant, parturient or puerperal women. 2. A markedly decreased volume of urine, an increase in the chloride concentration, and an average rise in the systolic blood pressure of 49 mm. Hg. in pre-eclamptic patients.

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## 8190 P

## Effect on Blood Pressure of Sudden Release of Intestinal Distention.

IAN AIRD.* (Introduced by E. A. Graham.)

*From the Department of Surgery, Washington University School of Medicine, and Barnes Hospital, St. Louis.*

A study has been made of the effect of changes in intestinal distention upon the blood pressure of a dog whose vagosympathetic trunks are divided and common carotid arteries ligated on both sides of the neck, and whose splanchnic nerves are divided. The blood pressure in such an animal would be expected to vary inversely with the degree of splanchnic congestion induced by the distention of the gut. The only factor difficult to control in such an animal is the contractility of the intestinal muscle, which in responding to a rise in intra-intestinal pressure may contract tightly, rendering the intestine anemic, and, by the consequent increase in peripheral resistance to the blood flow, causing a rise in blood pressure. This pressor effect obtained in 5 dogs in which the whole small intestine, ligated at both ends as a closed loop, was distended with air. In 5 dogs and 3 cats, however, the intestine remained passive under distention, and became distinctly cyanosed at an air pressure of 60-90 mm. of mercury, and the blood pressure of these animals exhibited at once a sharp fall of from 20-90 mm. of mercury within half a minute. Release of this intestinal distention after a period of from 5 minutes to 7 hours was followed by a return of the color of the bowel to normal, and by a rapid rise of blood pressure to its original level or beyond it.

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* Rockefeller Traveling Fellow from the University of Edinburgh.

In a further series of 5 dogs, prepared in the same way, the distention was maintained from 6-18 hours. Release of the distention was again followed by return of the bowel to a normal color, but in 3 animals, instead of the rise in blood pressure which occurred in the previous series, a sharp fall was observed. In one of these 5 dogs indeed the blood pressure, perfectly stable before release of the distention, fell progressively after release till the animal's death 7½ minutes later.

In 2 other animals distended for more than 18 hours, and in one animal distended for only 12 hours, the bowel failed to resume its normal color after collapsing and remained cyanosed. In these dogs there was no alteration in the blood pressure. The bowel was obviously devitalized and thrombosis of the vessels in its wall probably prevented the return of venous blood from the area of splanchnic congestion to the general circulation.

The unexpected fall in blood pressure on relief of an intestinal distention of 6-18 hours' duration strongly suggested the diffusion of a depressor substance from the lumen or from the tissue spaces of the bowel wall into the stagnating blood of the congested intestinal capillary bed, and the return of that substance after deflation with the portal blood to the general circulation, to exercise then its depressor effect. An investigation of this depressor was, therefore, commenced. It was first determined that normal dog portal plasma had no depressor effect. In 2 animals the portal vein was drained immediately after release of a 16-hour distention at a pressure of 80 mm. of mercury. The portal plasma of both these animals exercised a depressor effect upon the blood pressure of the intact dog. The depressor activity of the plasma was lost by dialysis through a celloidin membrane. Precipitation of the proteins of the plasma was performed by adding trichloracetic acid to a concentration of 10%, and the filtrate freed of trichloracetic acid by repeated ether extraction in a separating funnel. The protein-free filtrate so obtained had a depressor effect on the blood pressure of a dog in a dose comparable to 3 cc. of the active plasma. A similar protein-free filtrate of portal plasma from a normal dog was inactive. The protein-free filtrate failed to activate, as histamine would activate, the virgin guinea pig uterus or the isolated cat jejunum—its action on the rabbit's blood pressure and circulation have not yet been determined, but intraperitoneal injection in the young guinea pig was not followed by death, or by obvious respiratory difficulty. The filtrate had no action on frog rectus abdominis or on eserinized leech muscle, both of which contract with acetylcholine. The filtrate had no effect upon isolated rabbit intestine, which is depressed by adenosine and stimulated by the "substance P" of Euler and Gaddum. The diffusible depressor substance apparently present in the portal blood after release of an intestinal distention of 12-18 hours' duration thus remains unidentified.

### 8191 P

## Species-Specificity in Production of Anti-Gonadotropic Substances.*

C. F. FLUHMANN.

*From the Department of Obstetrics and Gynecology, Stanford University School of Medicine.*

The work of Collip and his collaborators[1] has shown that the prolonged daily administration of gonadotropic extracts to rats leads to the production of substances which can inhibit the action of such hormones. The present study was conducted to determine if an "anti-hormone" developing from the injection of an extract made from the pituitary glands of one species is effective against an extract prepared from the hypophysis of another species.

A series of 39 female rats, age 21 to 23 days at the beginning of the experiment, was injected daily with a weak dose of a gonadotropic extract prepared from human pituitary glands in the manner described previously.[2] In 5 days the ovaries had increased 50% in weight and showed developing graafian follicles and corpora lutea. The ovaries of the rats sacrificed in 30 days gave an 86% increase in weight, but in 60 days they were equal to those of the controls, and in 90 and 119 days they weighed slightly less than those of controls of the same age.

The blood serum of the rats sacrificed in 90 and 119 days was examined for the presence of "anti-hormones" according to the method used by Collip and Anderson[3] in testing for an anti-thyrotropic hormone.

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* Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Collip, J. B., *J. Mt. Sinai Hosp.*, 1934, **1**, 28.

[2] Fluhmann, C. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1193.

[3] Collip, J. B., and Anderson, E. M., *Lancet*, 1934, p. 76.

A mixture of equal amounts of a human pituitary gland extract and serum from the injected rats failed to produce any increase in ovarian weight and no histologic changes when given twice daily for 4 days to 4 immature rats. However, the same extract mixed with serum from normal animals produced an average of 618% increase in weight when given in the same manner to 3 immature rats. It was apparent that the injected rats had developed the power of inhibiting gonadotropic extracts prepared from human pituitary gland material.

The serum from the injected rats also had "anti-hormone" properties against gonadotropic extracts made from the blood of pregnant women. A mixture of serum from the treated rats and a human pregnancy blood extract failed to induce any histologic change and no increase in weight in the ovaries of 4 immature rats injected as before. The control series of 4 immature rats, when given a mixture of the same extract and serum from normal rats, showed a 190% increase in ovarian weight and the presence of developing follicles, corpora lutea, and lutein cysts.

On the other hand, the serum of the treated rats was ineffective in inhibiting gonadotropic extracts made from sheep pituitary glands. A mixture of serum from the rats given human pituitary extract for 119 days and a sheep pituitary gland preparation produced extensive histologic changes and an average increase in ovarian weight of 210% in the ovaries of 5 immature rats. In the same way, a mixture of the same sheep extract and serum from normal rats produced an average increase in ovarian weight of 300% in 5 immature rats.

It seems, therefore, that there is a definite species-specificity in the development of "anti-hormone" properties in the blood of rats injected for long periods of time with pituitary gonadotropic preparations.

### 8192 C

# Relation of Potency of Anterior Pituitary-Like Hormone to Hydrogen ion Concentration.

C. A. ELDEN AND MARVEL-DARE FELLOWS. (Introduced by J. R. Murlin.)

*From the Department of Obstetrics and Gynecology, University of Rochester School of Medicine and Dentistry.*

In a previous publication[1] concerning the separation of the anterior pituitary-like hormone from the urine of pregnant women by adsorption and elution, it was noted that the separation was dependent upon H-ion concentration. Therefore, it was decided to adjust the urine of pregnant women to various H-ion concentrations and determine the potency. The present communication deals with this relationship.

The method of procedure was to collect a mixed sample of the urine of pregnant women from the obstetrical clinic. The H-ion concentration was determined electrometrically using the hydrogen and calomel electrodes, and the urine was standardized in the following manner. Rabbits were injected in the marginal ear vein at 4:30 in the afternoon, with amounts varying in tenths of a cubic centimeter, and a laparotomy done the next morning to examine the ovaries. The smallest amount in tenths of a cubic centimeter causing the graafian follicles to rupture, in 3 out of 5 test animals, was then considered as the standard of potency of the fresh sample. Aliquots of the original mixed sample were adjusted to varying H-ion concentrations in the alkaline and acid range with a few drops of 18 normal sodium hydroxide or concentrated hydrochloric acid, and the exact pH determined as before.

The results are shown graphically in the accompanying curve where the pH is plotted against the cubic centimeters necessary to bring about a rupture of the graafian follicles. There is a range between a pH of 6.1 and 7.3 where the potency is greatest, with the curve rising in the form of a parabola from a pH of 7.3 to 12.0; and from a pH of 6.0 to 3.0. No ruptured follicles were found at any time with as much as 5 cc. at a pH of 12.0.

In order to determine whether the hormone potency was partially destroyed or inhibited, one collection was divided into 3 parts. The first having a pH of 6.48 was standardized. The second part was made acid with concentrated hydrochloric acid to a pH of 3.85, left one day and brought back to a pH of 6.12 with 18 normal

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[1] Elden, C. A., *J. Biol. Chem.*, 1933, **101**, 1.

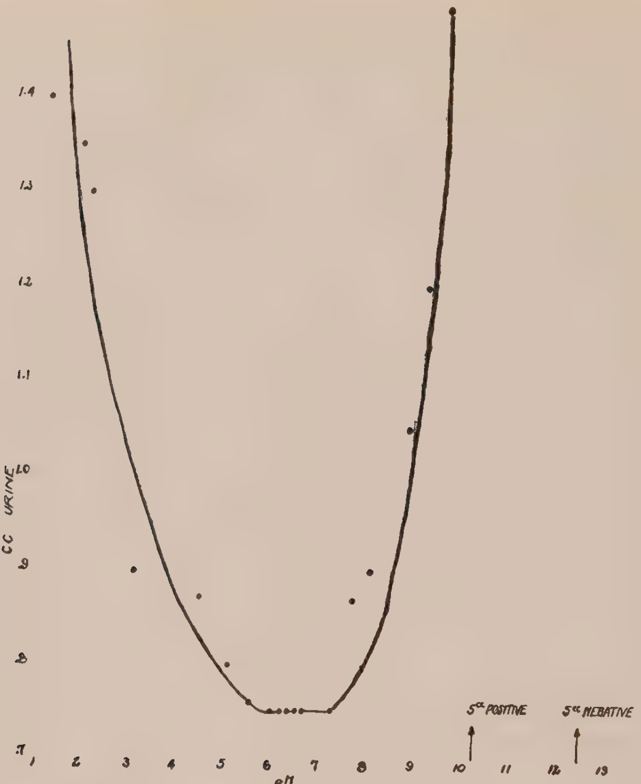

FIG. 1.

sodium hydroxide, where the potency was found to be unchanged. The third portion was made alkaline to a pH of 8.8, left 2 days, and adjusted to a pH of 5.0 where it was found to have decreased 50% in potency. Other samples which became more alkaline on standing for as long as 3 months in the icebox, and were then readjusted to the pH of maximum potency, were less affected than those artificially made alkaline with concentrated sodium hydroxide, and readjusted as above.

Zondek, *et al.*,[2, 3] have reported the best precipitation at a pH of 4.0. The findings in this investigation show the hormone to be most potent at a pH of 6.1 to 7.3. It seems highly probable that adsorption and elution may take place best at this pH; however it is possible that adsorption alone may take place equally well or even better at a different H-ion concentration, which would tend to keep

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[2] Zondek, B., and Aschheim, S., *Klin. Woch.*, 1928, **7**, 831.

[3] Zondek, B., *Zent. Gynak.*, 1929, **53**, 834.

any foreign proteins in solution. In this case the hormone should be more readily removed from contaminating mixtures, and obtained in a more pure form.

The curve showing the effect of H-ion concentration on the potency of the urine of pregnant women suggests that the hormone may be protein-like in nature, and have an isoelectric point not unlike hemoglobin. If this is the true state, its physical and chemical properties, such as solubility in water, precipitation by ammonium sulphate, and the fact that it is most potent at a pH which may well be its isoelectric point, confirm the facts. On the other hand the hormone may be adsorbed on a protein, and the various urinary proteins may have varying isoelectric points, where different amounts of the hormone are carried down.

It has been noted by some observers[1, 2, 3] that the biuret test has been negative, perhaps due to the dilute concentration of hormone present. However, since most observations have been done on rather crude preparations, with the protein as a possible contaminant, the validity of these statements awaits further elucidation, with the preparation of a chemically pure hormone.

*Conclusions.* From the foregoing results, conclusions are drawn that the urine of pregnant women is most potent in anterior pituitary-like action at a pH of 6.1 to 7.3. Potency is defined as the smallest amount of urine causing the graafian follicles to rupture in 3 out of 5 test animals. The hormone may be protein-like in nature, or carried down with the foreign protein at the isoelectric point of 6.1.

Potency is little affected by acid, but varies with the alkali content, sometimes being greatly reduced, as when sodium hydroxide is added, and again showing less change after 3 months in the icebox at an alkaline pH which has developed on standing.

These observations in no way affect the Friedman modification of the Aschheim-Zondek test,[4] since larger amounts varying from 5 to 10 cc. are always used in the latter.

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[4] Friedman, M. H., *Am. J. Physiol.*, 1929, **90**, 619.

8193 C

# Physiology of Pyrimidines. VIII. Metabolism of Isobarbituric Acid in the Rabbit.

WILLIAM J. CONWAY AND LEOPOLD R. CERECEDO. (Introduced by E. Brand.)

*From the Department of Chemistry, Fordham University, New York.*

As a preliminary to a study of the origin of ethereal sulfates in the rabbit, we thought it worth while to investigate the metabolism of isobarbituric acid in this species. In the adult dog[1] and in man[2] this pyrimidine is partly broken down to urea, partly conjugated with sulfuric acid.

Four adult rabbits were placed in metabolism cages, and kept on a diet consisting of equal parts of alfalfa and oats. The amount of food given each animal was 10 gm. less than that eaten at will. This was found to be sufficient to permit a slight gain in weight over a period of 3 months. The animals were allowed to drink water *ad libitum*. The freely voided urine was collected every 24 hours.

TABLE I.
Metabolism of Isobarbituric Acid in the Rabbit.

| | Day | Urine volume cc. | Total N gm. | Urea N gm. | Total S gm. | Inorganic S gm. | Ethereal S gm. | Neutral S gm. |
|---|---|---|---|---|---|---|---|---|
| Rabbit No. 1 | 1 | 158 | 1.76 | 1.17 | 0.203 | 0.133 | 0.012 | 0.058 |
| | 2 | 163 | 1.79 | 1.24 | 0.171 | 0.123 | 0.008 | 0.040 |
| | 3 | 165 | 1.82 | 1.22 | 0.190 | 0.128 | 0.011 | 0.051 |
| | 4 | 166 | 1.78 | 1.24 | 0.205 | 0.136 | 0.012 | 0.057* |
| | 5 | 171 | 1.96 | 1.50 | 0.202 | 0.086 | 0.058 | 0.058 |
| | 6 | 158 | 1.70 | 1.19 | 0.201 | 0.126 | 0.014 | 0.061 |
| | 7 | 162 | 1.79 | 1.10 | 0.196 | 0.131 | 0.013 | 0.053 |
| Rabbit No. 2 | 1 | 113 | 1.45 | 1.30 | 0.116 | 0.076 | 0.010 | 0.030 |
| | 2 | 121 | 1.43 | 1.11 | 0.121 | 0.078 | 0.012 | 0.031 |
| | 3 | 103 | 1.44 | 1.19 | 0.120 | 0.083 | 0.011 | 0.026* |
| | 4 | 112 | 1.66 | 1.36 | 0.150 | 0.043 | 0.069 | 0.038 |
| | 5 | 133 | 1.31 | 1.04 | 0.148 | 0.091 | 0.016 | 0.041 |
| | 6 | 127 | 1.44 | 1.23 | 0.143 | 0.094 | 0.009 | 0.040 |
| Rabbit No. 3 | 1 | 178 | 1.32 | 0.87 | 0.151 | 0.095 | 0.012 | 0.044 |
| | 2 | 153 | 1.29 | 0.91 | 0.154 | 0.098 | 0.013 | 0.043 |
| | 3 | 146 | 1.21 | 0.90 | 0.151 | 0.105 | 0.011 | 0.035* |
| | 4 | 160 | 1.52 | 1.11 | 0.164 | 0.027 | 0.093 | 0.044 |
| | 5 | 178 | 1.31 | 0.90 | 0.146 | 0.099 | 0.009 | 0.038 |
| | 6 | 153 | 1.20 | 0.84 | 0.121 | 0.083 | 0.008 | 0.030 |

* 1.5 gm. of isobarbituric acid fed with the food, N = 0.33 gm.

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[1] Cerecedo, L. R., *J. Biol. Chem.*, 1930, **88**, 695.

[2] Stekol, J. A., and Cerecedo, L. R., *J. Biol. Chem.*, 1933, **100**, 653.

Isobarbituric acid was prepared according to Davidson and Baudisch.[3] The purity of the compound was checked by analysis.

The following analytical methods were used: Total N, Kjeldahl; urea, Van Slyke's gasometric method[4]; inorganic and ethereal sulfur, Folin's method[5]; total sulfur, Denis' modification of Benedict's method.[6]

Isobarbituric acid was fed altogether 9 times to rabbits. Three representative experiments are given in Table I. We find a rise in the urea output, a drop in the inorganic sulfur fraction, and a corresponding increase in the ethereal sulfates. These observations indicate that the metabolism of isobarbituric acid in the rabbit follows the same path as in the adult dog and in man.

### 8194 C

## Serum Phosphatase in Normal Young Rabbits.

PAUL D. ROSAHN.

*From the Laboratories of the Rockefeller Institute for Medical Research.*

In connection with studies on certain familial bone abnormalities of the rabbit, the sera of animals presenting the deformities under investigation were analyzed for their phosphatase content. For comparative purposes similar determinations were made on a group of normal animals, and these normal findings form the basis of the present report.

The rabbits varied in age from 15 to 71 days, with a mean age of 39.3 ± 15.3 days. They were all vigorous, healthy young, with no clinical evidence of bone lesions, and were derived from lines unrelated to the group with bone pathology. All were caged with their mother or foster-mother. The diet consisted of maternal milk supplemented in varying degrees depending on the age of the animal by a mixture of grains and grain products, with alfalfa, mineral salts and a molasses binder. The technique employed in the serum phosphatase determinations was that described by Bodansky.* [1] Except

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[3] Davidson, D., and Baudisch, O., *J. Biol. Chem.*, 1925, **64**, 619.

[4] Van Slyke, D. D., *J. Biol. Chem.*, 1927, **73**, 695.

[5] Folin, O., *J. Biol. Chem.*, 1905, **1**, 131.

[6] Denis, W., *J. Biol. Chem.*, 1910, **8**, 401.

* The author wishes to thank Dr. Bodansky for personally demonstrating the technique of this procedure.

[1] Bodansky, A., *J. Biol. Chem.*, 1932, **99**, 97; *ibid.*, 1933, **101**, 93.

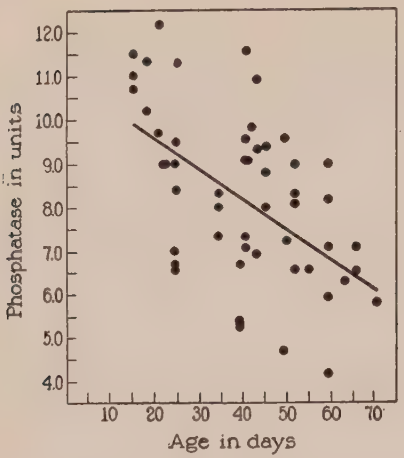

FIG. 1.

The relation between phosphatase and age in healthy young rabbits. $r_{pa}$ = —0.5373 ± 0.0986. Regression equation: $x$ = 10.90 — 0.067A.

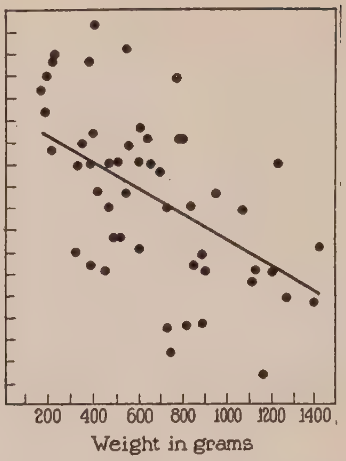

FIG. 2.

The relation between phosphatase and weight in healthy young rabbits. $r_{pw}$ = —0.5894 ± 0.0905. Regression equation: $x$ = 10.46 — 0.0032W.

$x$ = estimated value for phosphatase. A = age in days. W = weight in grams.

in a few instances where the quantity of serum was insufficient for the purpose, all determinations were made in duplicate.

The results are shown in the familiar scatter diagrams; in Fig. 1 they were plotted against age, and in Fig. 2 against weight. The mean value for the 53 determinations was 8.3 ± 1.9 units. It is seen that the phosphatase values decrease with increasing age and weight. The relationship between phosphatase and age was found to give a significantly negative correlation coefficient of —0.5373 ± 0.0986. A significant negative correlation coefficient of —0.5894 ± 0.0905 was also obtained between serum phosphatase and weight. The difference between these 2 coefficients is statistically insignificant.

The regression equation for phosphatase on age was $x$ = 10.90 — 0.067A, where $x$ is the estimated value for units of serum phosphatase, and A is age in days. This equation was employed in plotting the points of the solid line shown in Fig. 1. That it is applicable only within the age limits of the group included in the analysis was shown by determinations on 9 normal rabbits 12 and 13 days old. A mean value of 17.2 ± 2.4 units was obtained, whereas the equation predicts a significantly lower value of 10.0 units. In addition to this disparity between actual and predicted values in animals younger than those shown in the scatter diagram, differences were also noted between observed and predicted values in older animals. A few determinations on adult animals indicate that when growth ceases, the phosphatase values tend to remain at a constant level ranging between 4 and 8 units.

*Summary.* Within the age limits of 15 and 71 days the serum phosphatase values for healthy rabbits describe a statistically significant linear decrease with increasing age and weight.

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### 8195 P

## Effect of Spontaneous and Induced Infections upon Development of Mouse Sarcoma 180.*

GREGORY SHWARTZMAN.

*From the Laboratories of Mount Sinai Hospital, New York.*

It has been recently observed that bacterial factors capable of eliciting the phenomenon of local skin reactivity to bacterial filtrates also produce, upon intravenous injections, selective hemorrhagic necrosis in transplantable malignant tumors of guinea pigs, mice and rats (Gratia and Linz,[1] Shwartzman and Michailovsky,[2] Duran-Reynals,[3] and Apitz.[4] The work described deals with the effect of spontaneous and induced bacterial infections upon the development of mouse sarcoma 180.

The antagonistic and inhibitory effect of Trypanosomes, *Bacillus tuberculosis, Bacillus bulgaricus thermophilicus* and *B. anthrax* upon the development of mouse and rat transplantable tumors was previously reported by Daels,[5] Karczag, Csaba and Nemeth,[6] Roskin and Exempliarskaia,[7] and Boccolari-Segolini.[8]

*Growth of Sarcoma 180 in normal mice.* In a healthy stock of

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* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

[1] Gratia and Linz, *C. R. Soc. Biol.*, 1932, **108**, 427.

[2] Shwartzman, G., and Michailovsky, Nicholas, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 737.

[3] Duran-Reynals, D., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 341.

[4] Apitz, Kurt, *Z. f. Krebsforst.*, 1933, **40**, 50.

[5] Daels, F., *Arch. Hyg.*, 1910, **72**, 257.

[6] Karczag, L., Csaba, M., Nemeth, L., *Z. f. Krebsforst.*, 1931, **33**, 371.

[7] Roskin, G., Exempliarskaia, E., *Z. f. Krebsforst.*, 1931, **34**, 628.

[8] Boccolari-Segolini, A., *Ateno parmense*, 1933, **5**, 315.

mice the tumor gives 100% of takes. Within the first 2 weeks following inoculation the mortality rate is low (7 to 8%). According to Woglom, no fluctuations in growth energy and no seasonal influences on the growth of the tumor are observed. Based on inoculations of thousands of mice in the Crocker Institute for Cancer Research, spontaneous cure takes place in approximately 1.33%. In the experiments of the author of this paper, 2 weeks after the inoculation, about 50% of mice showed tumors measuring from 1.5x2 cm. to 2x2 cm. (large tumors) and the remaining, tumors measuring from 1x1 cm. to 1x1½ cm. (average tumors).

*Growth of Sarcoma 180 in mice infected with B. enteritidis.* Observations were made on a group of 610 mice infected spontaneously with *B. enteritidis.* Twenty-five and four-tenths per cent died during 12 days following tumor inoculation. Cultures of the tumor tissue, and pleural fluids, hearts' blood and liver of the dead and many of the surviving mice showed *B. enteritidis.* Two weeks following inoculation of the tumor, 377 surviving mice were examined. Of these no mice showed large tumors; 46% showed average tumors, 31% showed tiny necrotic tumors, and 23% no tumors. When mice bearing tiny necrotic tumors survived for 3 to 4 weeks following the transplantation, spontaneous regressions were observed in most of these. The exact percentage of regressions cannot be given since most of infected mice died within the first month. The possibility remains that the tumors would have resumed growth if the mice lived longer. It may be stated, however, that spontaneous regressions were observed beyond normal expectancy.

*The effect of induced bacterial infections on Sarcoma 180.* Young cultures of *Staphylococcus aureus* and of a rough variant of *B. enteritidis* respectively, were injected intravenously into mice bearing 12-days-old sarcoma 180. *Staphylococcus aureus* had no effect. *B. enteritidis* elicited hemorrhagic necrosis in tumors of 15 out of 20 mice tested within 24 hours. Eight of these tumors completely regressed. *Staphylococcus aureus* culture filtrate had no effect. *B. enteritidis* culture filtrate produced results similar to those obtained with live cultures of *B. enteritidis.*

Both filtrates were tested for their ability to elicit the phenomenon of local skin reactivity to bacterial filtrates in rabbits. *B. enteritidis* filtrate was active in dilution 1 to 500. *Staphylococcus aureus* filtrate produced no effect even in large doses.

As is seen from the above observations spontaneous and induced infections with *B. enteritidis* exert a striking inhibitory influence upon the development of sarcoma 180. There exists a definite correlation between the ability of the filtrate of a given microorganism to elicit the phenomenon of local skin reactivity in rabbits and the inhibitory effect of an infection with the microorganism upon the development of sarcoma 180 in mice.

Attempts are under way to determine the effect of infections with certain avirulent microorganisms upon the growth of animal and human spontaneous tumors.

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## 8196 P

## Changes in the Hypophyses of Castrate and Cryptorchid Guinea Pigs.*

WARREN O. NELSON.

*From the Department of Anatomy, Yale University, and the Hull Zoological Laboratory, the University of Chicago.*

Engle[1] has shown that the anterior hypophysis of the castrate rat is more potent in gonad-stimulating hormone than that of the normal animal. Evans and Simpson[2] confirmed this and demonstrated that glands from cryptorchid rats have more than normal potency. Severinghaus[3] showed that the same holds for the castrate guinea pig.

In the present experiments anterior lobes from normal and castrate (4 to 12 months) adult male and female, and experimental cryptorchid (6 to 14 months) guinea pigs were implanted intramuscularly into immature female rats and mice. In all, 235 animals were used as donors and 83 as hosts. The relative impotency of the guinea pig pituitary in gonadotropic hormone made it necessary to implant considerable amounts in the rat. The amount of guinea pig anterior lobe (fresh gland weight) required to induce a significant increase in the immature rodent ovary was found to be approximately 4 times that of rat pituitary. In the case of the five types of guinea pig pituitaries mentioned above from 3 to 5 times as much, by weight, was required in the rat as in the mouse. Similar

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* Aided by grants from the National Research Council, Committee for Research in Problems of Sex. Grants administered by Dr. E. Allen and Dr. F. R. Lillie. The phases of the study carried on at the University of Chicago were under the writer's tenure of a National Research Council Fellowship.

[1] Engle, E. T., *Am. J. Physiol.*, 1929, **88**, 101.

[2] Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1929, **89**, 371.

[3] Severinghaus, A. E., *Am. J. Physiol.*, 1932, **101**, 309.

results have been seen following the administration of sheep pituitary extracts.[4]

It was shown that the ovarian response following implantation of equal amounts of normal male and female pituitaries is approximately 50% greater in the case of the male. Pituitaries from castrate and cryptorchid males and spayed females were of equal potency, about 70% greater than normal male glands, in each instance. Pituitaries from females subjected to extreme partial castration were only slightly less potent than those from totally spayed animals. Glands from gonadectomized males and females receiving daily injections of estrin (25 to 100 R.U.) and from castrate and cryptorchid males bearing functional ovarian grafts were somewhat less potent than normal pituitaries.

The cytological changes in the guinea pig hypophysis following castration are not as clearly delineated as in the rat where a marked increase in the basophiles and an appearance of signet-ring castration cells occurs. Fichera,[5] and others, reported an increase of acidophiles in the castrate guinea pig. Severinghaus,[3] and Nelson[6] on the basis of a series of two-month castrates, found no noticeable increase in basophiles. In the present study pituitaries from castrates of longer duration have shown slight, but unmistakable increases in the percentage of basophiles. This increase does not attain the magnitude observed in the hypophysis of the castrate rat and is not accompanied by the appearance of vacuolated castration cells. The closest approximation to such cells which has been observed is the finding in some of the long time castrates, both male and female, of a few small vacuoles in the cytoplasm of the basophiles. Perhaps the most striking change seen in the hypophysis of the castrate guinea pig is a marked occurrence of cell nests arranged in a manner strikingly similar to the follicles of the thyroid gland. These "follicular" nests regularly contain a colloid-like coagulum. Although a tendency toward the occurrence of these structures is occasionally seen in the normal gland it is far less pronounced than in the castrate cryptorchids.

Hypophyses from cryptorchid were found to be similar to those of castrates, the changes developing somewhat more slowly. This has previously been shown to be true in the rat.[9] Castrates and cryptorchids bearing functional ovarian grafts have essentially normal pituitaries although many of the basophiles show evidence of degranulation. This degranulated condition of the basophiles is more pronounced in castrates and normals receiving daily injections of estrin. These findings confirm the earlier work[7, 8] concerning the effect of estrin on the basophiles of the rat hypophysis.

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[4] Nelson, W. O., and Overholser, M. D., *J. Pharm. and Exp. Therap.*, 1935, in press.

[5] Fichera, G., *Arch. Ital. Biol.*, 1905, **43**, 405.

[6] Nelson, W. O., *Am. J. Anat.*, 1933, **52**, 307.

[9] Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1192.

The changes in the guinea pig hypophysis in castrate, cryptorchid, and estrin-injected animals are seen to be of the same character, although less marked, as those found in the rat. This study serves as a basis of interpretation for the results following ovarian transplantation in guinea pigs of both sexes.

## 8197 P

## Presence of Anti-Pernicious Anemia Principle in Normal Human Urine.*

GEORGE E. WAKERLIN.

*From the Department of Physiology and Pharmacology, University of Louisville School of Medicine.*

In a previous communication[1] we reported a significant modification of the pigeon method of Vaughan, *et al.*,[2] for the laboratory assay of anti-pernicious anemia preparations and showed that normal human urine contains a principle which significantly increases the reticular material in the red blood cells of the pigeon in a manner analogous to the anti-pernicious anemia principle present in liver. At the same time, we reported the preparation of a urinary extract which had a similar effect. In order to test for the identity of the urinary and liver principles, we indicated our plan to study the effect of the urinary extract in pernicious anemia. Due to the well-known present difficulty in securing pernicious anemia patients suitable for assay work, this preliminary report deals with the results obtained with the extract in one patient.

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[7] Nelson, W. O., *Anat. Rec.*, 1933, **55**, 70 (suppl.).

[8] Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 452.

* This work was aided by a grant from the Therapeutic Research Committee of the American Medical Association.

[1] Wakerlin, G. E., Bruner, H. D., and Kinsman, J. M., *Proc. Am. Physiol. Soc.*, 1935, pg. 136.

[2] Vaughan, J., Muller, G. L., and Zetzel, L., *Brit. J. Exp. Path.*, 1930, **11**, 456.

The extract was prepared by concentrating urine to a small volume *in vacuo* at a temperature of 37°C., adding sufficient 95% ethanol to give a concentration of 70%, concentrating the resulting filtrate *in vacuo* to a small volume at 37°C., adding sufficient 100% ethanol to give a concentration of 95%, collecting and drying the precipitate, and finally dissolving it in physiological salt solution containing 0.5% phenol. Enough saline solution was used to give a 1:20 ratio for the volumes of the extract and the original urine respectively, although a smaller amount of the solvent would have sufficed. This method is basically that commonly employed in the preparation of parenteral liver extracts.

The volume of this urinary extract necessary to increase significantly the reticular material of the pigeon is approximately equal to that for one commercial parenteral liver extract (3 cc. equivalent to 100 gm. of liver), slightly less than that for another (2 cc. equivalent to 100 gm. of liver), and definitely less than that for a third (20 cc. equivalent to 100 gm. of liver). Blood pressure studies on the rabbit showed the urinary extract to contain a somewhat lower concentration of depressor substances than the parenteral liver extracts just mentioned. The rabbit was found to tolerate a dosage of 5 cc. per kilo of the urinary extract administered intravenously. Larger amounts were not used. Subcutaneous and intramuscular injections of the extract (previously tested for sterility) into the rabbit produced no gross evidence of chemical inflammation.

The patient† employed in this study was first admitted to the Louisville City Hospital in September, 1933, when 45 years of age, with typical clinical and laboratory findings of pernicious anemia, inclusive of early cord changes. Oral liver therapy induced a regression of his signs and symptoms with an improvement in his blood picture from 1,600,000 R.B.C. and 47% Hb. (Sahli) to 3,200,000 R.B.C. and 68% Hb. by the eighteenth day. A maximal reticulocytosis of 23% was observed on the eighth day of therapy. During his subsequent 20 days in the hospital, the patient's R.B.C. and Hb. remained essentially unchanged despite a daily treatment regimen of 500 gm. of liver by mouth, supplemented by 3 cc. of liver extract (equivalent to 100 gm. of liver) intramuscularly. Although oral liver therapy was continued for a time following discharge from the hospital, a subsequent observation period of 2 months in the Outpatient Department failed to show any significant change in the blood picture. The patient, therefore, was less responsive than the average to liver treatment.

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† The patient was made available for study through the courtesy of Dr. John Walker Moore of the Department of Medicine of the University of Louisville School of Medicine.

For 12 months prior to his return to the hospital in April, 1935, the patient had taken no form of anti-pernicious anemia treatment. On this admission a blood study showed R.B.C., 2,650,000; Hb. (Newcomer), 63%; reticulocytes, 0.6%; and W.B.C., 3,500. Largely on the basis of the comparative dosage data obtained from the pigeon assay of the urinary extract cited above, the patient was given a total of 14 intragluteal injections of 3-6 cc. of the urinary extract (each equivalent to 60-120 cc. of urine) during a 35-day period of treatment. The injections were well-tolerated locally and generally. The individual doses and their spacing are recorded in Fig. 1. Iron and ammonium citrate, 6 gm. daily, was also administered during the latter part of the treatment period in view of the patient's tendency to oscillate about a subnormal blood level.[3] Iron alone, of course, is well-known to be without effect in pernicious anemia. No other therapy was given. As seen from Fig. 1, there was a gradual and slightly irregular increase in the R.B.C. and Hb. to levels of 3,640,000 and 83%, respectively by the thirty-fifth day of treatment with the urinary extract. It should be noted that these levels were definitely higher than those previously attained with liver therapy. The W.B.C. also showed a gradual increase to 6,100. A maximal reticulocyte count of 4% occurred on the fourth day. This mild reticulocytosis is, of course, usual with initial

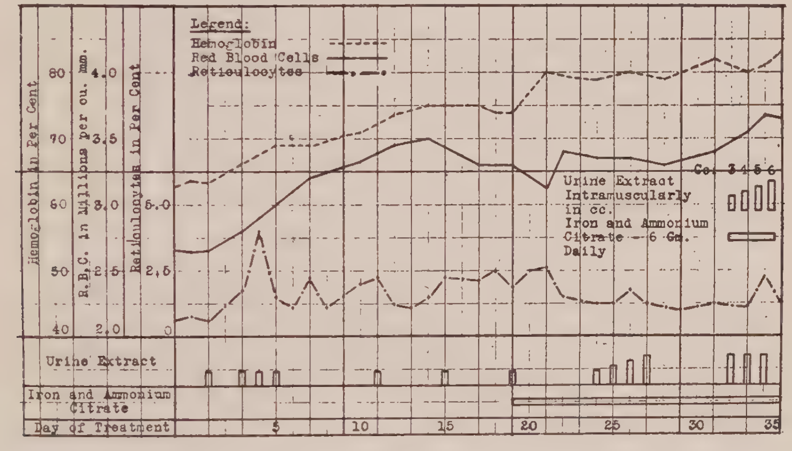

FIG. 1.
The effect of injections of the urinary extract on a patient with pernicious anemia.

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[3] Beebe, R. T., and Lewis, G. E., *Am. J. Med. Sc.*, 1931, **181**, 796.

R.B.C. counts approaching 3,000,000. Coincidentally with the change in his blood picture, the patient was subjectively and objectively improved.

Verification of these results in additional and more suitable pernicious anemia patients with lower blood findings will be attempted as soon as they are available. Arrangements have also been made for trial of the extract in two clinical laboratories. The preliminary data presented, however, suggest that the anti-pernicious anemia principle is present in normal human urine, apparently in sufficient amounts to warrant consideration of the latter as a commercial source for the principle.

## 8198 C

## Absence of Follicle-Stimulating Hormone in Pituitaries of Young Pigeons.

OSCAR RIDDLE and JAMES P. SCHOOLEY.

*From Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.*

Earlier studies of Riddle[1, 2] showed that in young pigeons and doves the growth rate of testes is very low during a period of several weeks after hatching; and that during this period of slow testis growth a very rapid body growth is almost completed. Reckoning age from the beginning of embryonic development the curves obtained for testis growth[2] showed that in common pigeons the testes begin a marked increase in growth rate at 2.3 months (1.7 mo. after hatching); in ring doves this spurt in testis growth occurs at about 3.0 months (2.5 months after hatching). The slow growth of testes during the period of rapid body growth was interpreted as evidence that dove and pigeon pituitaries—unlike those of mammals—do not release the gonad-stimulating hormone until the more advanced ages mentioned above; also that the more advanced age at which the testes begin their spurt of growth probably coincides with the initiation of F.S.H. secretion in the bird's pituitary. This interpretation seems to be further supported by the unmatched sensitivity of the immature dove testis to true follicle-stimulating hormone (F.S.H.) administered to these birds. In other words, the great sensitivity of

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[1] Riddle, O., *Am. J. Physiol.*, 1928, **86**, 248.

[2] Riddle, O., *Endocrinology*, 1931, **15**, 307.

these immature testes suggests that they have not previously been subjected even to traces of F.S.H. derived from their own pituitaries. Direct tests, by the implant method, for the presence or absence of F.S.H. in the pituitaries of immature and mature pigeons were needed to settle this question and such tests are reported here.

Immature male ring doves, aged 2.2 to 2.7 mo., with average body weight of 156 gm., were used as hosts for the implants. The weight of untreated testes of these dove hosts, at the age at which they were sacrificed, can be known to be approximately the same as the average testis weight obtained for a series of control birds of exactly the same age and race; in our dove races the individual deviations from such an average weight only slightly exceed one-half of the average value. The donor pituitaries were obtained from the much larger common pigeons, immature birds in some tests, mature birds in others. In 2 tests, made for an interesting comparison, the donors were either immature or young adult white rats. All results are given in Table I.

It will be noted that one rat pituitary per day for 10 days, or for 9 days, produced significant testis growth, 100% and 300%, respectively. Also one adult pigeon pituitary, from males or from females, for only 5 days gave increases of 100% and 200%; the data suggest a greater potency of the female pituitary. On a 10-day basis—with a relatively much greater response expected from prolonged dosage—an increase of 800% was obtained from one adult female pigeon (or from 2 adult ring doves) pituitary implanted daily. In the 2 tests of *immature* pigeon pituitaries, these donors having almost attained adult body size, the resulting testis size in the recipient is within or almost within the variable limits of normal unstimulated testis size. Both, however, are near the upper limit and it is entirely probable that one or more of the older of the 60 donors for these 2 tests had already made their first beginnings of F.S.H. secretion. The data obtained on the gonad weights of certain of these donors confirm this suspicion. In order to obtain 60 immature donors slightly above 1.8 months old, it was necessary to use 4 such donors of border-line age (2.5-2.6 mo.) among the group of 30 supplying implants for dove No. VII, and the gonad weights of 2 of these 4 donors indicate some F.S.H stimulation from their own pituitaries (*i. e.,* their gonads had started their spurt of growth); among the group of 30 donors for No. VIII there were 7 birds of this border-line age, and the gonad weights of 4 of these indicate that they had already entered into their period of rapid growth. Despite the presence of these few slightly older donors

TABLE I.

Doves Implanted with Pituitaries from Rats and Pigeons; F.S.H. Detectable (Increased Testis Weight) in All Implants Except Those from Immature Pigeons.

| Data on recipient doves |  |  |  | Data on donors |  |  |  | Testis weight |  |
|---|---|---|---|---|---|---|---|---|---|
| No. | Age (mo.) | Days implants given | No. of pituitaries implanted | Kind and condition | Total body weight (kilos) | Total pituitary weights (mg.) | Recipient crop-gland weight (mg.) | Expected if untreated (mg.) | Actually found (mg.) |
| I | 2.7 | 10 | 10 | Rats, ♂, 29-44 days | 0.53 | 22.5 | 450 | 8.4 | 17.3 |
| II | 2.5 | 9 | 9 | ” ♂, 60-110 ” | 1.19 | 40.0 | 335 | 8.2 | 27.4 |
| III | 2.4 | 5 | 5 | Pigeons, ♂, mature | 2.33 | 21.0 | 290* | 7.8 | 15.9 |
| IV | 2.3 | 5 | 5 | ” ♀, ” | 2.17 | 23.4 | 270 | 7.4 | 21.4 |
| V | 2.2 | 10 | 17† | ” ” | 3.44 | 34.6 | 365 | 6.8 | 60.2 |
| VI | 2.5 | 10 | 30 | ” ” | 13.15 | 128.4 | 395 | 7.4 | 66.2 |
| VII | 2.3 | 10 | 30 | ” 1.8-2.5 mo. | 11.22 | 122.0 | 390 | 7.0 | 9.2 |
| VIII | 2.5 | 10 | 30 | ” 1.8-2.6 mo. | 10.82 | 108.9 | 455* | 8.2 | 13.6 |

* Note an increase in crop-gland size parallel with number of pituitaries implanted. In view of the extensive experience of this laboratory with unstimulated crop-gland weights this parallelism may be taken as very strong evidence of the presence of prolactin in the pituitary of the pigeon—including that of the immature pigeon. The 10 immature rat pituitaries also yielded a crop-gland (No. I), whose weight and appearance indicate some prolactin stimulation.

† On each of 7 days 2 mature ring doves were used instead of one mature pigeon.

(whose gonads were just beginning to show an accelerated rate of growth) both of the values obtained from hosts given a group of 30 implants are such as to show that F.S.H. is not detectable in the pituitaries of 30 common pigeons aged 1.8 to 2.3 months. By the same method of testing—and despite the relatively slighter effect of short-term dosage—F.S.H. is detectable in fewer than 5 implants of adult pituitaries.

The small total amounts of immature rat pituitary tissue which were found effective may be compared in Table I with the large amounts of immature pigeon pituitary tissue which were ineffective. Total body weights are also given for all groups of donors. Of the 60 immature pigeon pituitaries transplanted 31 were from males and 29 were from females.

The results obtained from implants of pigeon pituitaries thus confirms the conclusion earlier drawn from curves describing testis growth in common pigeons; the similar conclusion drawn from the growth curve of the dove testis therefore receives indirect experimental support. This experimental evidence is important in current studies of the anterior pituitary. Until or unless 2 available kinds of evidence are overthrown it must be granted that doves and pigeons of a defined stage of immaturity are "without a pituitary" in so far as the F.S.H. is concerned. In these birds no pituitary hormone or extract now known may be expected either to decrease or to increase an output of F.S.H. from pituitary cells which have not yet started their production of F.S.H. Incidentally this latter circumstance provides the probable explanation of the inability of some Prolan, P.U. and A.P.L. preparations to affect the immature bird testis. By using these intact animals—and avoiding hypophysectomy with its heavy assault upon the whole organism—one can yet study the action of all A.P. hormones in the absence of endogenous follicle-stimulating hormone.

*Summary.* The testes of immature ring doves are measurably stimulated by implants of 1 immature (29-44 days) rat pituitary daily for 10 days; one adult common pigeon pituitary daily for 10 days gives much greater stimulation; stimulation can be obtained from fewer than 5 adult pigeon pituitaries; 30 pituitaries of immature but nearly full grown pigeons (1.8 to 2.3 months from beginning development) implanted in groups of 3 daily for 10 days give no detectable stimulation. These results confirm an earlier indication from growth rate of testes in these birds: Pigeons less than 2.3 months old (1.8 months after hatching) and ring doves of less than 3.0 months (2.5 months after hatching) are to be considered as animals in which the production of F.S.H. has not yet been initiated. When anterior pituitary hormones, or extracts of this tissue, are injected into these birds it must be granted that their action is obtained on an intact organism free from follicle-stimulating hormone, and that the injected material serves neither to reduce nor to induce an output of F.S.H. from the bird's own pituitary.

### 8199 C

## Effects of Complete and Incomplete Hypophysectomy on Basal Metabolism of Pigeons.*

OSCAR RIDDLE, GUINEVERE C. SMITH, AND CLARENCE S. MORAN.

*From the Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.*

The use of pigeons in the assay of anterior pituitary hormones makes it desirable to measure and record the effect of hypophysectomy upon the basal heat production of these animals. Earlier data for the dog, recently reviewed by Houssay,[1] indicate an average decrease of 16% as a result of this operation; in the rat —35% is reported by Foster and Smith,[2] and —26 by Collip.[3] The present measurements were made at 2 environmental temperatures—one series being done at the critical temperature for the pigeon (30°C.), the other at 20°C. The data obtained show that the percentage change in B.M.R. following complete hypophysectomy in the pigeon is much influenced by the environmental temperature at which the measurements (pre- and post-operative) are made. Measured at 30° such pigeons show a 33% decrease; at 20° the decrease is only half that amount, or 17%. That these differences are not chance and spurious resultants of few measurements made on variable material is further attested by much unpublished data dealing with the capacity of individual anterior lobe hormones to affect the B.M.R. of hypophysectomized pigeons at these 2 temperatures.

Adult pigeons of various races were used. At 10-20 days after operation the completely hypophysectomized pigeons had lost about 25% of their previous body weight. The post-operative metabolism measurements were made 10-20 days after the operation, in a mul-

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* Aided by a grant from the Carnegie Corporation of New York.

[1] Houssay, B. A., *Endocrinology*, 1934, **18**, 409.

[2] Foster, G. L., and Smith, P. E., *J. A. M. A.*, 1926, **87**, 2151.

[3] Collip, J. B., *J. Mt. Sinai Hosp.*, 1934, **1**, 28.

tiple-chamber apparatus, with technique earlier described;[4] this includes housing the birds during their 24-hour fasting period in a large constant temperature glass cage kept at the same temperature at which the B.M.R. is to be measured. The data were obtained from February to August. In only a few birds was a comparison made of the rectal temperatures at pre- and post-operative periods; in these cases no definite change in body temperature was noted. At autopsy the sella and its contents were examined in serial microscopic sections, except in 3 cases observed only under dissecting microscope. When traces of anterior pituitary tissue were found, measurements and calculations were made to determine the percentage of tissue present. These histological studies provide the classification used for summary presentation in Table I; and suggest that anterior pituitary tissue in the pigeon has no regenerative power. Some pigeons with minute pituitary fragments had been operated as much as 10 months before autopsy.

TABLE I.
Effects of Complete and of Partial Hypophysectomy on the Basal Heat Production of Common Pigeons.

| Completeness of operation | No. of tests | Calories per kilo per hr. before operation | Percentage decrease of B.M.R. after hypophysectomy — Min. | Max. | Aver. |
|---|---|---|---|---|---|
| | | Measurements made at 30° C. | | | |
| Complete | 9 | 3.86 | 13 | 50 | 33 |
| 0.3-4.0% in | 7 | 3.78 | 15 | 43 | 28 |
| 10-23% in | 5 | 3.96 | 18* | 40 | 18 |
| | | Measurements made at 20° C. | | | |
| Complete | 7 | 4.29 | 1* | 36 | 17 |
| 1.3-10.0% in | 4 | 4.36 | 13 | 31 | 19 |
| 13-20% in | 4 | 4.19 | 4 | 41 | 19 |

* These values are +18 and +1.

The data permit a tentative statement concerning effects of partial hypophysectomy on the B.M.R. It is found, in measurements made at 30°, that when less than 4% of the gland is present there is little or no difference from complete hypophysectomy; 10-20% of this tissue usually effect a partial but incomplete restoration of the pre-operative B.M.R. In our measurements made at 20° the metabolism is decreased to an equal extent irrespective of whether the hypophysectomy was complete or incomplete. The results obtained in the 30°C. tests practically parallel Smith's findings on the capacity of graded amounts of pituitary tissue to sustain the structural normality of thyroids, adrenals and gonads in partially hypophysectomized rats.[5]

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[4] Benedict, F. G., and Riddle, O., *J. Nutr.*, 1929, **1**, 497.

[5] Smith, P. E., *Anat. Rec.*, 1932, **52**, 191.

*Summary.* Within 10-20 days after complete hypophysectomy the B.M.R. of the adult pigeon undergoes a pronounced decrease, but the extent of the indicated decrease is much influenced by the environmental temperature at which measurement is made. At the critical temperature (30°C.) the B.M.R. of 9 pigeons was decreased by 33%; in 7 pigeons measured at 20°C. the decrease was only half as much, or 17%. Seven incompletely hypophysectomized birds (less than 4% of pituitary present) measured at 30° provide data which confirm the important rôle of temperature and further indicate that the B.M.R. of such birds is practically the same as that of the completely hypophysectomized pigeon. Pituitary fragments representing 10-25% of the total gland usually only partially sustain the normal rate of heat production. B.M.R. measurements made at 20° did not distinguish partial from complete hypophysectomies, and this provides a further indication that a truer measure of effect of hypophysectomy is obtained from tests made at the animal's critical temperature.

## 8200 P

## Heparin as an Anticoagulant in the Brucella Phagocytic Index Test.

L. VEAZIE AND K. F. MEYER.

*From the George Williams Hooper Foundation, University of California, San Francisco.*

In a previous publication,[1] the application of the Brucella phagocytic index test to the study of epidemiology of undulant fever was discussed. Tests carried out on over 1,000 people seemed to indicate that the test was of value in the detection of passed, latent and present infections with the Brucella organisms.

When the same technic was applied to the natural hosts, goats and cattle, the differences between the normal and the infected animals were not striking. Although positive indices were obtained in some of the smaller laboratory rodents, in larger animals the phagocytic activity of the leucocytes, despite the presence of Brucella organisms in the tissues, was either absent or very low. The indices of the infected or immunized animals were, however, consistently high, when, instead of whole citrated blood, the sedimented cells thoroughly washed and resuspended in Locke's gelatin solution were mixed with fresh serum and a bacterial suspension in equal quantities. From these observations it was concluded that the citrate solution was toxic for the leucocytes of certain herbivorous animals or inhibited in some way the phagocytic activity of the granulocytes. Since the washed cell method was too time-consuming for routine use, it was decided to search for an anticoagulant that would be more suitable for the purpose. Several were tried, including sodium oxalate, sodium fluoride, "Liquoide", amniotic fluid, and heparin. Heparin* was found to be far the most effective. A 1-1,000 dilution of heparin in saline (1 drop of blood to 1 drop of heparin solution) for human blood and 0.2 cc. of a 1:200 dilution for cattle and goats was employed, the test being conducted otherwise exactly as with citrate.

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[1] Meyer, Stewart, Veazie, and Eddie, PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 284.

In Table I the significant data are summarized. A comparison of the indices determined on the leucocytes of citrated and heparinized blood clearly indicates that cattle as well as goats yield much higher indices when heparin is employed as an anticoagulant. In a group of recently infected cows the average index of the granulocytes in citrated blood was 1.6 and in heparin-treated blood it was 13.6. A herd with a record of freedom from abortion disease for over 6 years yielded one animal with an index above 5 with heparinized cells. Blood specimens of goats revealed the same differences. In fact, the existence of an infection was, as a rule, reflected by the index figure provided the blood specimen had been treated with heparin. An experimental group of goats infected and in various stages of latent Malta fever had always furnished specimens with negative phagocytic indices with citrate but showed highly active leucocytes and indices from 1 to 18 in the heparinized blood.

In a series of 100 human beings with active or latent Brucella infections, in which the phagocytic tests were carried out in duplicate, using both citrate and heparin, the differences between the 2 anticoagulants were not so apparent, though the average index in the heparin series was somewhat higher, being 5.8 as against 4.3 for citrate. Furthermore, in rare instances the blood of a febrile patient with a negative history for undulant fever gave with heparin an index figure suggestive of a latent infection. During convalescence and after recovery the index invariably dropped to the level typical for a normal human being.

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* Since this anticoagulant had been tested experimentally for over 8 months, Boerner and Mudd, *Am. J. Med. Sc.*, 1935, **189**, 23, independently recommended its use.

TABLE I.
Comparative Phagocytic Indices in Blood Specimens Treated with Heparin and Sodium Citrate.

| | Serologically (agglutination and complement fixation) and allergically positive | | | | | Serologically and allergically negative | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species | No. tested | Aver. heparin index | Range | Aver. citrate index | Range | No. tested | Aver. heparin index | Range | Aver. citrate index | Range |
| Goats | 27 | 7.0 | 1.8-18.6 | 0.7 | 0 - 2.7 | 21 | 1.1 | 0-4.3 | 0.3 | 0-0.5 |
| Cattle | 10 | 13.6 | 7.5-21.8 | 1.6 | 0 -10.8 | 30 | 3.0 | 0-5.08 | 0.07 | 0-1.0 |
| Guinea pigs | 8 | 13.6 | 4.3-27.6 | 4.8 | 0.4- 8.4 | — | — | — | — | — |
| Humans | 100 | 5.8 | 0 -27.3 | 4.3 | 0 -24.0 | — | — | — | — | — |
| Incubation | | | | | | | | | | |
| 30 min. | 12 | 8.4 | 0 -27.3 | 3.4 | 0 -11.6 | | | | | |
| 60 ’ | 12 | 13.4 | 0 -35.9 | 5.5 | 0 -19.4 | | | | | |

It should be stressed that the inaccuracies inherent to the method are numerous, and too great importance should not be placed on minor variations in the index. Although experience has taught that false positives are quite uncommon, a negative result should not be accepted as final until 2 or 3 specimens of blood have been taken at varying intervals. A trace of acid in a tube, too long an interval between the collection of the blood and the addition of the organisms, a slight alteration in the pH of the reagents, partial clotting of the blood, or the admixture of tissue juice when the blood is secured by puncture of the ear or finger instead of the vein may lower or entirely prevent phagocytosis of the Brucella organisms. The length of the incubation period at 37°C. alters the index markedly in many instances, the number of bacteria ingested being often twice as great at the end of an hour as after 30 minutes. Whether there is a difference in the susceptibility of leucocytes to external influences which may change from day to day is not known, but it has been noted that blood taken from the same person on different days, though the conditions of the test were as nearly identical as possible, gave widely differing results. It is recommended that when possible, tests be run in duplicate, using both heparin and citrate as anticoagulants, until the factors controlling the phenomenon are more fully understood.

## 8201 P

## Action of Carbaminoylcholine on the Iris, Normal and Parasympathectomized.

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The ciliary ganglion and its accessories were removed from one orbit in each of a series of cats. At intervals thereafter, the effects on the pupils of a drop of carbaminoylcholine chloride solution in each eye, were studied in the dark-room under conditions of uniform lighting. In the experiments here reported, the dosage in each eye was equivalent to 0.3 mg. of carbaminoylcholine chloride and the eyes, when studied, were 60 cm. from a screen of white muslin, illuminated by a 100 watt mazda lamp, 40 cm. away.

When the irises were tested *within 24 days* after the operation, the pupils in both the normal and the parasympathectomized eyes were constricted down to the same size, approximately 1 mm. in horizontal diameter. The constriction began within 5 minutes, in both eyes, reached its maximum, in both eyes, at about 45 minutes and, in most cases, remained at this maximum more than 45 minutes.

Since it is probable that the nerve fibers and their endings had completely degenerated before the end of 14 days, and since Armstrong[1] has shown that acetylcholine does not depress the nerveless heart, the foregoing facts suggest that carbaminoylcholine in the given concentration, stimulates only the parasympathetic neuromuscular junctions.

In the later months after the operation, the drug in the normal iris still constricted the pupil down to a 1 mm. width, but in the parasympathectomized iris, it constricted the pupil only to about 8 mm. These findings in the later months are probably due to the degeneration of disuse, either in the denervated parasympathetic neuromuscular junctions or in the muscle fibers.

## 8202 P

## Observations on Intra-Intestinal Pressure with Special Reference to Absorption of Saline.

ROBERT ELMAN AND IAN AIRD.* (Introduced by E. A. Graham.)

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Measurement of the absorption of fluid from an isolated fistulous loop of intestine by the use of an ordinary manometer tube is unsatisfactory, since with absorption the manometer level falls progressively, and with it, the intra-intestinal pressure. The intra-intestinal pressure can, however, be maintained at a constant level by a manometer system built on principles similar to that used by White[1] for the measurement of the intracapsular pressure in the kidney. Inserted in a closed loop of small intestine previously prepared, is a rubber catheter, which is encircled close to its eye by an inflatable rubber balloon, which serves as a flange fitting snugly in the fistulous passage through the abdominal muscles and preventing escape of fluid. The catheter is attached by a glass T tube to a long rubber connection, continuous in its turn with a fine glass tube. This glass tube, open at its other end, is kept horizontal at any required level above the loop. The system is filled with physiological saline by syringe through the third limb of the T tube, until the fluid reaches the horizontal glass tube, forming a meniscus there which is brought to a mark near the center of the tube. Movement of the meniscus away from this mark toward the rubber connecting tube indicates absorption of fluid from the system, movement towards the open end of the glass tube indicates secretion by the isolated bowel loop. The amount of fluid added to the system by syringe over a given period to hold the meniscus steady at the mark on the horizontal tube will now allow measurement of the rate of absorption from the intestinal loop. Peristaltic movements of the isolated loop, never very considerable, were greatest with each change in pressure but decreased to a stable level after 10 minutes. No readings were recorded during the first 10 minutes of each pressure period.

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[1] Armstrong, P. B., *J. Physiol.*, 1935, **84**, 20.

* Rockefeller Traveling Fellow from University of Edinburgh, Scotland.

[1] White, H. L., *Am. J. Physiol.*, 1928, **85**, 191.

The rate of absorption of physiological saline has been measured by this method in 3 dog loops of ileum (8 to 12 inches in length) at various levels of intra-intestinal pressure. Anesthesia was unnecessary for these observations. In none of these animals did an appreciable absorption occur at a pressure of less than 250 mm. As the intra-intestinal pressure increased, so at first did the rate of absorption, to reach a maximum of 48 cc. per hour at 700 mm. of water in the first loop, 96 cc. per hour at 660 mm. in the second, and 125 cc. per hour at 500 mm. in the third. At higher pressures, the rate of absorption steadily fell, to reach zero at 900, 850, and 850 mm. of water in the respective loops. When the intra-intestinal pressure was still further raised beyond these levels, movement of the meniscus towards the open end of the horizontal tube indicated secretion by the loop mucosa. These observations were repeated and confirmed in the case of each loop.

An opportunity presented itself of making a similar series of observations on human intestine. A female patient in the course of a series of operations for a complicated small intestine obstruction, presented at one stage of her treatment an exposed 6-inch jejunal loop, completely isolated from the remainder of the bowel. The point of maximum absorption (8 cc. per hour) occurred at an intra-loop pressure of 400 mm. of water, and thereafter the rate fell till a pressure of 700 mm. was attained. At pressures of 800 mm. or more, definite secretion occurred into the loop. At 800 mm.

the loop became obviously cyanosed, and at that pressure only did an uncomfortable sensation of fullness ("like gas") occur. The peristaltic movements of the loop, occurring with each change in pressure, were never excessive, and did not prevent accurate centralization of the meniscus. The pulse beat was transmitted to the fluid in the system.

The curve of absorption in these experiments was a straight line in agreement with the observations of Wells,[2] who found that absorption of saline from closed loops was directly proportional to the intra-intestinal pressure. Wells's studies, however, included pressures up to 160 mm. water only.

The same patient at an earlier stage of her treatment presented a jejunal fistula. This fistula was efficiently drained by a tube which bore an inflatable balloon collar identical with that described for the experiments in dogs. By attaching this drainage tube to a manometer, a record was made of the spontaneous changes in intra-intestinal pressure. The pressure base line was 20 to 40 mm. throughout the course of the experiment. On 3 occasions, during 30 minutes, the pressure rose rapidly to 150, 250 and 275 mm. of water. Each of these excursions lasted 30 to 60 seconds. Minor waves up to 60 or 70 mm. were twice as frequent. With nearly all these upward excursions of the manometer, there was a complaint of mild hypogastric discomfort. Moderately intense umbilical colic was experienced whenever the pressure reached 200 mm. of water, this threshold level being exceedingly constant throughout the whole course of the experiment, and also observed on several other occasions in the same patient. These excursions were more frequent and more pronounced after the ingestion of water and of light food, reaching a peak on one occasion of 350 mm. of water.

## 8203 P

## Dissimilation of Pyruvic Acid by Lactobacillus lycopersici.

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*From the Department of Bacteriology, Iowa State College, Ames, Iowa.*

In view of the intermediary rôle assigned to pyruvic acid in bacterial metabolism, the mechanism of its breakdown by *Lactobacillus*

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[2] Wells, H. S., *Am. J. Physiol.*, 1931, **99**, 209.

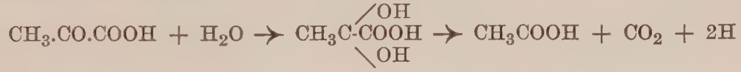
*lycopersici* is of importance to an understanding of the dissimilation of carbohydrates by the heterofermentative lactic acid bacteria.

A medium containing pyruvic acid, peptone, yeast extract and $K_2HPO_4$ adjusted to pH 6.2 was fermented anaerobically at 30°C. by *L. lycopersici.* Formation of the 3 final products in equimolar quantities (Table I) indicates a dissimilation involving oxidation of one molecule of pyruvic acid and the simultaneous reduction of a second. Decarboxylation of pyruvic acid does not appear to occur since fixation of acetaldehyde by dimedon or bisulphite was repeatedly unsuccessful. The close relationship of the lactic bacteria to the propionic bacteria and the failure of van Niel[1] and Wood and Werkman[2] to detect acetaldehyde in fermentations by the latter suggests application of the proposal of these investigators to the lactic acid bacteria, *i. e.,* breakdown of pyruvic acid according to equation (1). The active hydrogen serves to reduce a second molecule to lactic acid.

TABLE I.
Dissimilation of Pyruvic Acid by *Lactobacillus lycopersici.**

| | Exp. 1 | Exp. 2 |
|---|---|---|
| | mm. per liter | mm. per liter |
| Pyruvic acid, fermented | 70.5 | 46.7 |
| Lactic acid | 37.7 | 22.8 |
| Acetic acid | 35.1 | 22.6 |
| $CO_2$ | 38.8 | 23.8 |

*Millimols per liter.



$$CH_3.CO.COOH + H_2O \rightarrow CH_3\underset{\diagdown OH}{\overset{\diagup OH}{C}}\text{-}COOH \rightarrow CH_3COOH + CO_2 + 2H \qquad (1)$$

## 8204 P

# Effect of a Deficient Diet, Amytal and Amidopyrine on Blood Picture of the Albino Rat.

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*From Iowa State College.*

Madison and Squier[1] in a discussion of the etiology of primary granulocytopenia report that in their series of 14 patients the onset was preceded in every case by the taking of amidopyrine combined with a barbiturate. Also they report the absence of granulocytes in a rabbit given these drugs. In light of this report it seemed worth while to test the action of amytal and amidopyrine on the blood picture of rats weakened by a deficient diet.

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[1] van Niel, C. B., The Propionic Acid Bacteria, Thesis, Delft, 1928.

[2] Wood, H. G. and Werkman, C. H., *Biochem. J.*, 1934, **28**, 745.

[1] Madison, F. W., Squier, T. L., *J. Am. Med. Assn.*, 1934, **102**, 755.

Rats of the Ames-Wistar strain, about 60 days in age, were used. The percentage composition of the diet was: Commercial casein 18; corn starch 54; butter fat 18; lard 6; and Osborne and Mendel salt mixture 4. Sodium amytal and amidopyrine 0.75 gr. each (initial dosage which is just sublethal) were freshly dissolved in water and administered daily to each rat by stomach tube, the animals being anesthetized with ether. Untreated litter mates were fed the standard laboratory ration. Total white counts and Hb determinations were made every 10 days, using U. S. Bureau of Standards pipettes and counting chambers and the Bausch and Lomb colorimeter. Differential counts, necropsies, and bone marrow examinations were made on all animals.

TABLE I.
Blood Findings.

| | No. of animals | | Duration of treatment | —Initial— | | Treated animals—Final | | Untreated animals—Final | |
|---|---|---|---|---|---|---|---|---|---|
| Exp. | Treated | Untreated | days | W.B.C. | Hb | W.B.C. | Hb | W.B.C. | Hb |
| I | 6 | 1 | 20 | 15,400 | 13.5 | 7,000 | 13.0 | 16,600 | 14.0 |
| II | 3 | 2 | 65 | 10,500 | 13.0 | 3,700 | 13.0 | 11,100 | 14.5 |
| III | 5 | 3 | 32 | 11,100 | 13.5 | 7,100 | 16.0 | 13,100 | 14.3 |
| IV | 4 | 2 | 42 | 11,100 | 13.5 | 5,900 | 14.0 | 12,100 | 13.5 |
| V | 8 | 2 | 34 | 13,500 | 15.0 | 8,600 | 15.0 | 14,300 | 16.0 |
| Total | 26 | 10 | | | | | | | |
| Ave. | | | 38.5 | 12,800 | 13.7 | 6,450 | 14.2 | 13,500 | 14.5 |

Stomatitis did not occur in these rats; differential counts showed granulocytes throughout the period and no significant reduction in the percentage of these cells. The bone marrow findings were not different from the normal litter mates. The rats showed hematuria continuously after the first few days' treatment. They became very weak and emaciated. Rats fed the deficient diet with no drugs showed no significant decrease in W. B. C. Necropsies showed no gross internal lesions.

*Summary.* The white blood count of rats, weakened by a deficient diet, may be reduced approximately 50% by oral administration of amytal and amidopyrine. Granulocytes do not disappear from the blood stream.

## 8205 C

# Ascorbic Acid Content of Blood.

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While numerous papers have appeared dealing with the ascorbic acid content of various plant and animal tissues, and urine, only a few refer to the quantity present in the blood. Van Eekelen, Emmerie, Josephy and Wolff,[1] and Emmerie and Van Eekelen[2] deproteinize blood, blood plasma, or serum with trichloracetic acid, precipitate interfering substances, chiefly SH compounds, with mercuric acetate, then treat with $H_2S$, which not only precipitates the mercury but also reduces that portion of ascorbic acid which in blood occurs in a reversibly oxidized state. The $H_2S$ is later removed by a stream of nitrogen, and the ascorbic acid estimated by titration with 2:6 dichlorobenzenoneindophenol.[3] Gabbe[4] claims that loss of ascorbic acid occurs if a solution of pure ascorbic acid is treated with $H_2S$ in the presence of mercuric acetate, and therefore, omits this step. Tauber and Kleiner[5] describe a method generally applicable to plant and animal tissues (and to blood) in which the essential features of deproteinization, removal of interfering substances, and $H_2S$ reduction are preserved. They estimate the ascorbic acid present either by titration or by its ability to reduce potassium ferricyanide, with subsequent development of Prussian-blue upon the addition of the ferric gum ghatti reagent of Folin and Malmros.[6]

Our experience with several of these methods disclosed a number of difficulties. Some of the methods require considerable quantities of blood. Mercuric acetate solutions must not be over two weeks old, or filtrates containing colloidal sulphides may be obtained, particularly when the method is applied to urine. Any colloidal material in the filtrate makes it impossible to obtain a satisfactory endpoint during the titration with the dyestuff. The endpoint with trichloracetic acid filtrates is not sharp.

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[1] Van Eekelen, M., Emmeric, A., Josephy, B., and Wolff, L. K., *Klin. Wschr.*, 1934, **13**, 564.

[2] Emmerie, A., and Van Eekelen, M., *Biochem. J.*, 1934, **28**, 1153.

[3] Tillmans, J., Hirsch, P., and Hirsch, W., *Zeitschr. f. Untersuch. d. Lebensmittel*, 1932, **63**, 1.

[4] Gabbe, E., *Klin. Wschr.*, 1934, **13**, 1389.

[5] Taüber, H., and Kleiner, E. S., *J. Biol. Chem.*, 1935, **108**, 563.

[6] Folin, O., and Malmros, J., *J. Biol. Chem.*, 1929, **83**, 115.

In the present study, we are chiefly concerned with finding some method whereby the relative ascorbic acid level of blood in infants and children may be determined as an aid in studying subclinical scurvy and following its course under treatment. Since the amount of blood obtainable is frequently limited, the following method has been employed: Five or more cubic centimeters of blood are drawn and oxalated. The blood is centrifuged immediately, the plasma removed, and a tungstic acid filtrate prepared. Usually 2 cc. of plasma are pipetted into a round bottom 15 cc. centrifuge tube, 4 cc. distilled water added, followed by 2 cc. of 5% sodium tungstate, the contents mixed, then 2 cc. of ⅓ normal sulphuric acid added, in the usual manner.[7] After *thorough* mixing, the tube is allowed to stand for 1 to 2 minutes, then centrifuged. About 6 to 7 cc. of clear fluid are obtained. Two cubic centimeter portions are pipetted into round bottom centrifuge tubes and titrated immediately to the first faint pink color (compared with the untitrated solution) as rapidly as possible. A 5 cc. microburette (div. 0.01 or 0.02 cc.) is used to measure the required volume of dye solution.

The dyestuff is prepared as follows: Approximately 0.1 gm. of sodium 2:6 dichlorobenzenoneindophenol (E. K. Co. No. P 3463) is twice extracted with scalding hot water (for maximum solution). Each extract is poured through a small filter paper, combined, and diluted with freshly boiled and cooled water to a volume of 50 cc. Reject the insoluble residue. This stock solution is good for about 3 weeks. Dilute 10 cc. of this stock solution to 100 cc. with boiled and cooled distilled water. This diluted solution after standardization is used for titration of the deproteinized plasma fluid.

The diluted dyestuff is standardized as follows: A definite amount (approximately 60 mg.) of ascorbic acid (Cebione, Merck, 0.1 gm. ampules, vacuum sealed) is weighed out and dissolved in 5% acetic acid solution (made from freshly boiled and cooled water) and diluted to 100 cc. Two cubic centimeters of this solution are transferred to a 50 cc. volumetric flask and diluted to the mark with 5% acetic acid solution. (This solution approximates in ascorbic acid content that of deproteinized plasma.) The dilute dyestuff is now titrated against 2 cc. portions of dilute ascorbic acid solution, in the manner prescribed for titration of the deproteinized plasma solution. We check the ascorbic acid solution further by titration against a 0.01 N iodine solution (1.14 cc. 0.01 iodine = 1 mg. ascorbic acid) which has previously been standardized against potassium iodate (Merck's Reagent). The ascorbic acid solution must be used immediately after standardization.

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[7] Folin, O., Laboratory Manual of Biological Chemistry, 5th Ed., Appleton and Co., 1934, p. 263.

Table I shows the recovery of various quantities of ascorbic acid added to the plasma before deproteinization.

TABLE I.
Recovery of Added Ascorbic Acid (on Basis of 2 cc. Deproteinized Plasma).

| Plasma | Initial Ascorbic Acid mg. | Added Ascorbic Acid mg. | Total Ascorbic Acid mg. | Recovered mg. | Recovered % of total A. A. |
|---|---|---|---|---|---|
| Dog* | .0045 | .1838 | .1883 | .1782 | 94.64 |
| ,, | .0045 | .1838 | .1883 | .1800 | 95.59 |
| Human | .0067 | .0010 | .0078 | .0074 | 93.25 |
| ,, | .0067 | .0021 | .0088 | .0076 | 86.36 |
| ,, | .0054 | .0008 | .0063 | .0063 | 100.00 |

* In this series 2 additional determinations were run on the same plasma deproteinized by means of trichloracetic acid. The recovery values were 102.28 and 97.29 %, respectively.

Ascorbic acid values of plasma deproteinized by trichloracetic acid (2 cc. plasma, 5.5 cc distilled water, 2.5 cc. 20% $CCl_3COOH$) have in many instances been determined on the same specimen as deproteinized by tungstic acid. The values in trichloracetic acid fil-

TABLE II.

| Student | Age, Yrs. | Ascorbic Acid | | Customary inclusion or absence of citrus fruit from diet before starting experiment |
|---|---|---|---|---|
| | | Urine mg./cc. Single Specimen† | Blood Plasma mg./100 cc. Tungstic Acid Filtrate | |
| B.I. | 32 | | | Oranges daily |
| Initial value | | 0.0068 | 1.375 | |
| O.J. for 1 week | | .1150 | 1.463 | |
| R.M. | 22 | | | Citrus fruit once a day |
| Initial value | | .0015 | 1.030 | |
| O.J. for 1 week | | .0022 | 1.350 | |
| A.N. | 21 | | | ,, ,, occasionally |
| Initial value | | .0041 | 0.687 | |
| O.J. for 1 week | | .0049 | 1.350 | |
| R.R. | 22 | | | 2 or more oranges daily |
| Initial value | | .0047 | 2.290 | |
| O.J. for 1 week | | .1200 | 2.360 | |
| F.S. | 21 | | | Occasional orange |
| Initial value | | .0011 | 0.916 | |
| O.J. for 1 week | | .0044 | 1.463 | |
| E.W. | 22 | | | Citrus fruit daily |
| Initial value | | .0016 | 1.145 | |
| O.J. for 1 week | | .0227 | 1.350 | |

† Estimated according to method of Harris and Ray, *Lancet*, 1935, **228**, 71.
O.J. = Orange juice.

trates are usually a few milligrams percent higher due to 2 causes: the endpoint of the titration is less definite, and trichloracetic acid apparently contains some foreign substance which reduces the dyestuff a little less rapidly than ascorbic acid.

Table II shows data obtained on a group of medical students receiving one pint of orange juice daily for one week.

Table III presents data taken at random on children attending the pediatrics clinic.

TABLE III.

| | | Ascorbic Acid | | |
|---|---|---|---|---|
| Name | Age, Yrs. | Urine mg./cc. Single Specimen | Blood Plasma mg./100 cc. Tungstic Acid Filtrate | Customary diet: fruits, vegetables |
| B.M. | 13½ | .0083 | 0.819 | Citrus fruit, green vegetables |
| E.R. | 1 | .0029 | 0.671 | None |
| ” | 1 | — | 0.513 | Diet unchanged |
| ” | 1 | — | 1.687 | After receiving 30 mg. Cebione (Merck) daily for 1 week |
| K.P. | 9 | .0450 | 1.374 | 1 orange daily |
| M.W. | 7 | .0111 | 0.859 | Green vegetables |
| E.M. | 14 | .0001 | 0.458 | No fruit or tomato juice for years. (Allergic asthma) |
| M.J. | 5 | .0112 | 1.220 | Orange or tomato daily |
| D.M. | 1 | .0049 | 1.168 | Orange daily |

Mirsky, Swadesh, and Soskin[8] in a recent study state that they have been unable to make any correlation between the *total* ascorbic acid content of blood and the dietary regime. Our data presented in the tables clearly indicate that the ascorbic acid percent in the blood in the *reduced* form follows closely the Vitamin C intake. Assuming the correctness of the observations of Mirsky, Swadesh, and Soskin, the conclusion is unavoidable that for clinical purposes the reduced ascorbic acid content of blood is of the greater significance. We are collecting additional data on this question.

Plasma deproteinized by tungstic acid cannot be treated with $H_2S$ in order to effect a reduction of any ascorbic acid occurring in the oxidized form. Such treatment leads to the formation of a colloidal yellow tungsten sulphide, which up to the present has been impossible to remove. We have, however, subjected trichloracetic acid deproteinized plasma to $H_2S$ reduction, and obtained enhanced ascorbic acid values. Several values are shown in Table IV.

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[8] Mirsky, A., Swadesh, S., and Soskin, S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1130.

TABLE IV.
Ascorbic Acid Values in mg. per cc. Plasma.

| Subject | D.N. | I.L. | V.W. | J.D. | J.F. |
|---|---|---|---|---|---|
| Tungstic Acid Filtrate<br>Ascorbic Acid in reduced form | 0.422 | 1.346 | 1.010 | 1.262 | 0.926 |
| Trichloracetic Acid<br>Filtrates after $H_2S$ reduction<br>Total Ascorbic Acid | 0.802 | 1.683 | 1.978 | 1.569 | 1.123 |

These data show no constant relationship between reduced and total ascorbic acid values in the various plasma samples.

In conclusion, we wish again to state that this method is presented with full knowledge that it estimates only that portion of ascorbic acid occurring in plasma in the reduced form. It requires but small amounts of blood, and as far as we have been able to determine to date, accurately indicates the nutritional state of the body relative to Vitamin C.

We wish to acknowledge our indebtedness to Merck and Co., Inc., for a grant supporting this investigation, and to Miss Elizabeth Smith for technical assistance.

### 8206 P

## Studies on Non-bacterial Cholecystitis. A Non-Traumatizing Technique for Study of Chemical Cholecystitis.*

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To produce an experimental cholecystitis from which conclusions can be drawn concerning human cholecystitis, 3 basic postulates must be fulfilled. First, the cholecystitis must be a direct result of the material used, and not even partially due to an inherent defect in the technique. Second, the material introduced must be such as is normally found either in the body fluids or in the ingested food, and should not be artificial and foreign to the body. Third, the reaction produced in the gall-bladder by this material must be similar in appearance to human cholecystitis.

The ideal method for introducing any substances into the gall bladder is by way of its natural passages, *e. g.*, the common duct.

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* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

This is possible by leading a catheter up into the gall bladder through an opening in the common duct as close to the duodenum as possible, to avoid any manipulation of the gall bladder. To reestablish a rather normal flow of bile, a glass cannula can be fixed in place in the common duct at the point of the opening.

The detailed technique is as follows: The experiments were performed on dogs under ether anesthesia. The abdomen is opened by a midline incision, and the duodenum lifted aside, exposing the common duct. The latter is freed from the surrounding tissue and an untied ligature put around the distal end. An unfolded sponge is inserted under the duct, thus isolating it from all surrounding structures and preventing any bile from flowing on to the peritoneum through the opening which is made. A longitudinal incision about 1 cm. long is now made in the common duct about 1.5 cm. from its entrance into the duodenum, the margins of this opening then being clamped on each side by a moskito forceps. A soft, French rubber catheter, 2.5 to 3.5 mm. in diameter, depending on the width of the duct, is introduced through this opening into the gall bladder. As much bile as possible is then aspirated by means of a syringe attached to the end of the catheter, and an equal amount of material is injected. To prevent a back flow of bile through the opening in the common duct while the catheter it being drawn out and the glass cannula inserted, it is necessary to clamp off the common duct for a short time during this process. This can be done very gently by making use of a rubber-protected clamp, placed as close as possible to the gall-bladder, to allow room for the cannula to be inserted.

The glass cannula which was used is 3 cm. in length and 4 mm. in diameter, tapering at each end and grooved a short distance from the tips on each side. The cannula is gently introduced through the duct opening and is so set in place that the central bulging portion, between the 2 grooves, is visible through the opening. The previously inserted ligature is then tied in place over the distal groove of the cannula and another ligature tied around the proximal grooved surface. The cannula is now held tightly in place. After unclamping the duct and observing a normal flow of bile towards the duodenum, the edges of the opening are sutured together above the cannula.

The above procedure is carried out without any difficulty in 60-70% of the cases. The entire process, including the introduction of the cannula, can be accomplished in 20-25 minutes, without even bringing the gall bladder into view, thus assuring freedom from traumatization. In about 20% of the cases, however, it is not possible to introduce the catheter successfully into the gall bladder without special manipulation. If the catheter encounters any great resistance about 6-8 cm. from the opening in the duct, it is fairly certain that it has entered one of the hepatic ducts. The catheter should then be withdrawn, and the particular hepatic duct clamped off. This procedure usually allows the catheter to proceed in the right direction, *i. e.*, into the gall bladder.

Control experiments were run to show that the technique of this operation does not produce any reaction or traumatization in the gall bladder. In some cases, bile was aspirated and nothing was injected, while in others saline was injected through the catheter as a control. In all instances, there was a complete absence of reaction in the gall bladder.

In summary, this operation can be accomplished in 60-70% of the cases without any traumatization, as the technique is quite simple. In 20% of the cases, there is necessary a special skill on the part of the surgeon which, too, can be quite readily mastered. The operation cannot be accomplished in only a very few per cent of cases, in which the common duct is too fine for even the smallest catheter to be introduced.

## 8207 P

## Studies on Non-Bacterial Cholecystitis. Protein Injection into Gall Bladder and Reaction of Gall Bladder in Anaphylaxis.*

H. G. ARONSOHN AND E. ANDREWS.

*From the Department of Surgery, the University of Chicago.*

In spite of the previous failure to find a correlation between the nitrogen content of the gall bladder and cholecystitis, it was thought advisable to approach the matter experimentally by increasing the nitrogen content and observing the effects. This was done by the injection of egg albumen.

Fifteen dogs were operated upon. The egg albumen solutions used were first the undiluted white of egg and second, a 6% solution in Ringer's of crystalline purified egg albumen. They were injected either directly into the gall bladder cavity or into the wall. In 9 of the 15 cases the introduction was done by means of a needle puncture and in 4 cases a catheter was introduced into the gall bladder through an opening in the common duct and the material introduced through this. In some instances either the common or cystic duct was ligated and in others the gall bladder was allowed to remain patent. The amount of egg albumen solution used was 6 cc. in every instance. Before the introduction of this material 6 to 10 cc. of bile were aspirated from the gall bladder. When the injection was made into the wall of the gall bladder, 6 or 8 different spots were injected with about ½ cc. either subserously or into the deeper layers. The dogs were electrocuted in 48 hours and cultures and sediment tests of the bile made as well as microscopic examinations of the gall bladder.

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*This work was done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

Thirteen of the 15 gall bladders showed active recent inflammatory processes in the wall. These were characterized by edema, fibrinous exudate and hemorrhage. The gall bladder contained thick, deep green bile clouded with large bile-stained albumen flakes. Histologically, in all of the cases inflammatory reaction was present. This was much more pronounced in the outer layers. The most constant finding was a marked edematous thickening of the serosa with round cell infiltration which was not so marked. The lymphatics were often greatly dilated and fibrinous deposits in and over the serosa were frequent. The mucosa was normal while a minimal round cell infiltration was seen in the muscularis. This histological picture resembles that described in man by Boyd,[1] Graham,[2] Feinblatt,[3] Andrews,[4] and Denton[5] much more closely than does cholecystitis produced by bacterial infection, although the picture is not as extreme as the experimental chemical cholecystitis produced by other agents to be reported later. That this reaction in the gall bladder is not due merely to the local mechanical effects of the needle puncture is obvious when one considers that the same results were obtained on the free surface or when the puncture was made through the hepatic surface or even when the material was introduced through a catheter in the common duct. Similarly the effects were not due to duct ligation alone since the same results were obtained in other instances where the connection was left patent.

The possibility was considered that this is an anaphylactic reaction and for this reason 2 dogs were given intravenous injections of egg albumen previous to the time of operation. The local reaction to egg protein, however, remained unchanged. Furthermore, other animals received 2 injections into the gall bladder after 3-day intervals and the effects observed were no greater than one would expect on a purely additive basis.

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[1] Boyd, Textbook of Surgical Pathology.

[2] Graham, Diseases of the Gallbladder and Bile Ducts.

[3] Feinblatt, H. M., *New England J. Med.*, 1928, **109**, 1073.

[4] Andrews, E., *Trans. West. Surg. Assn.*, 1934.

[5] Denton, T., *Arch. Surg.*, 1927, **14**, 1.

These experiments seem to indicate that the reaction obtained by injection of protein into the gall bladder is not anaphylactoid in character. Clinical observations, however, suggest the possibility that in man a reaction may occur on the biliary system based on an anaphylactoid condition.

Alvarez[6] has recently described 4 cases in which pain, apparently in the gall bladder, was due to an anaphylactic response against certain foodstuffs (chicken, milk and eggs), omission of these from the diet affording relief. A series of cases of very similar nature has recently been observed by Croutch.[7] However, the specific anaphylactic reaction of the gall bladder has never, as far as we know, been produced in the experimental animal. For that reason, the following experimental observations may be of interest:

Each of 3 dogs received 4 intravenous injections of crystallized egg albumen within a period of 3 days, each injection consisting of 5 cc. of a 6% solution. No particular reactions were observed either during or after these injections. After an interval of 6 days, there was injected again a 6% solution of the same crystallized egg albumen, but this time in much larger quantities, sufficient to produce visible and marked anaphylactic response. In 2 of the animals, the reaction consisted of vomiting, trembling, irregular respiration, and passage of feces, while in the third dog, the response was considerably more pronounced, with vomiting, loss of consciousness, cessation of respiration for 3 minutes, and generalized convulsions. The quantity of the last injection was 15-20 cc. in the first 2 dogs, and 25 cc. in the last. The animals were electrocuted one-half hour following the last injection, and post-mortem examination made immediately. In all 3 cases, there was found a marked edema of the gall bladder wall, more specifically of the serosa. The thickness varied from 3 to 6 mm. Microscopic examination of the liver revealed markedly constricted arterioles.

As a control experiment, egg albumen was injected in large amounts into 3 other dogs, which had not, however, received any previous injections. In 2 of these animals, no symptoms were visible until 40 cc. had been injected, while after 45 cc. the reaction was marked but consisted only of vomiting and passage of feces. In the third dog, these symptoms were not present until the injection of 60 cc. Electrocution was performed one-half hour later, and this time post-mortem examinations revealed perfectly normal gall bladders. A comparison of the results in the 2 series of animals leads us to the conclusion that the reaction obtained after the last injection in the first series was an anaphylactic response, since it required much less albumen to produce a response in the previously sensitized dogs than in the control animals. Furthermore, the constriction of the arterioles in the liver in the first series of animals is suggestive of anaphylactic change.

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[6] Alvarez, W. C., *Proc. Staff Meetings of the Mayo Clinic*, 1934, **9**, 45.

[7] Croutch, personal communication.

## 8208 C

## Effects of Phenacetin and Aspirin Respectively upon Action of Phenobarbital.

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Some practical advantages of combining an analgesic with a hypnotic drug have been suggested by the work of Loewe and others. In particular, Käer and Loewe[1] investigated the hypnotic and toxic effects of members of 2 different analgesic groups in combination with barbital, namely, aspirin and phenacetin.

Aspirin was found to antagonize to a similar extent both the hypnotic and toxic effects of barbital. Phenacetin, on the other hand, while also antagonizing the toxic effects of barbital, appeared to enhance the milder hypnotic effects of the latter drug. This would suggest a better "therapeutic ratio" for a mixture of barbital and phenacetin than for barbital alone. In addition, such a mixture would have the additional therapeutic advantage of supplementing the hypnosis with analgesia.

We have completed an investigation along this line in which aspirin and phenacetin have respectively been used in combination with another member of the barbiturates, namely phenobarbital.

Rats were used as experimental subjects. The animals were fasted 24 hours before the administration of the drugs which were given by stomach tube. Phenobarbital was administered in solution in the form of the magnesium salt. In those experiments in which an analgesic was added, it was suspended in 2% acacia in the phenobarbital solution. To make all experiments comparable, 2% acacia was also added to the magnesium phenobarbital. In all cases the volume administered was kept constant (1.0 cc./100 G. rat), increased dosage being accomplished by increasing the concentration of drug in solution or suspension. All narcotized animals were kept in a warm room (constant at 31°C.) to minimize heat loss while anesthetized.

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[1] Käer, E., and Loewe, S., *Arch. f. exp. Path. und Pharm.*, 1926, **116**, 140; 1926, **118**, 108.

Phenobarbital was administered at 2 levels of dosage, minimal and toxic, the lower level to determine the effect of the addition of an analgesic upon the minimal hypnotic dose, the higher level to ascertain the toxicity of the combination of drugs. The proportion of phenacetin to phenobarbital was 5:1 by weight, the analgesic being given in the higher dosage. This proportion represents the ratio of the average therapeutic dosage of the drugs.

*Results. Minimal hypnotic dose.* It was found that magnesium phenobarbital when fed orally to rats in amounts of 25 mg./kilo produced symptoms of excitement followed by slight hypnosis. The administration of 50 mg./kilo produced more pronounced effects, the animals remaining depressed for several hours.

The addition of acetyl salicylic acid in amounts equal to the phenobarbital proved definitely antagonistic. Thus animals receiving 50 mg./kilo of magnesium phenobarbital plus 50 mg./kilo of aspirin failed to show signs of any excitement or depression.

On the other hand, the combination of phenobarbital plus 5 times the amount of phenacetin behaved in exactly the same manner as the phenobarbital controls. Rats receiving 25 mg./kilo of magnesium phenobarbital plus 125 mg./kilo of phenacetin showed excitement and slight hypnosis, whereas double this amount produced a depression lasting for several hours.

*Toxic doses.* In view of the fact that phenacetin was in no way antagonistic to the hypnotic action of phenobarbital, a large series of animals was fed toxic amounts of these 2 drugs in the same 5:1 ratio to determine the effect of the addition of the analgesic upon the toxicity of phenobarbital. The results are summarized in Table I. As can be seen from the table, the combination of phenacetin and phenobarbital proved to be less toxic than phenobarbital alone.

No difference could be observed between the 2 groups of animals

TABLE I.
Comparative Toxicity of Magnesium Phenobarbital and Magnesium Phenobarbital Plus Phenacetin.

| Dose Mg. Phenobarbital mg./K | | No. Rats | Deaths 12 hr. | 24 hr. | 48 hr. | 72 hr. | Total | % |
|---|---|---|---|---|---|---|---|---|
| 200 | | 30 | 1 | — | 1 | — | 2 | 7 |
| 225 | | 30 | — | 5 | — | — | 5 | 17 |
| 250 | | 10 | 3 | — | 1 | — | 4 | 40 |
| 275 | | 10 | 3 | 2 | — | — | 5 | 50 |
| 300 | | 10 | 3 | 1 | — | 1 | 5 | 50 |
| Total | | 90 | 10 | 8 | 2 | 1 | 21 | 23.5 |
| | Phenacetin mg./K | | | | | | | |
| 200 | 1000 | 30 | — | — | 1 | — | 1 | 3 |
| 225 | 1125 | 30 | 3 | — | 1 | 1 | 5 | 17 |
| 250 | 1250 | 10 | 1 | — | 1 | — | 2 | 20 |
| 275 | 1375 | 10 | 2 | — | — | — | 2 | 20 |
| 300 | 1500 | 10 | 1 | 1 | — | — | 2 | 20 |
| Total | | 90 | 7 | 1 | 3 | 1 | 12 | 13.5 |

with respect to the time of onset of hypnosis, the duration of anesthesia, and the recovery period.

*Conclusion.* The above results confirm for phenobarbital those reported by Loewe for barbital. It is apparent that when an analgesic is given simultaneously with a hypnotic drug, care must be exercised in the choice of the former. Thus, whereas acetyl salicylic acid antagonizes the hypnotic action of phenobarbital, phenacetin in no way diminishes its activity. Furthermore, the evidence shows that phenacetin may exert a protective influence by antagonizing the toxic effects of phenobarbital.

## 8209 C

## Effect of Diet upon Blood Phosphorus Partition of Rats with and without Insulin.

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Beginning with the work of Harden and Young and followed by that of Embden and his associates, it has been evident that phosphorus may play some rôle in the oxidation of carbohydrate. This idea became more fixed after the isolation of insulin when it was shown that not only blood sugar but also urine phosphate and blood inorganic phosphorus decreased after the administration of this hormone. The drop in blood inorganic phosphorus following insulin injection has been observed by Wigglesworth, Woodrow, Smith and Winters[1] for normal rabbits, Briggs, Koechig, Doisy, and Weber[2] for normal dogs, and Harrop and Benedict[3] for normal and diabetic human beings.

Studying the different fractions of blood phosphorus during insulin action, Kay and Robison[4] found that, in the rabbit, there is little change in the lipoid phosphorus, an increase in total acid soluble, a slight decrease in the inorganic phosphorus, and therefore an increase in the organic portion. Kerr[5] reported, in a similar study on the partition of blood phosphorus in dogs under the influence of insulin, a drop in the inorganic phosphorus and variable changes in the lipoid fraction, although he was unable to find, as had Kay and Robison, an increase in the organic fraction.

The decrease of blood inorganic phosphorus after insulin injection has been taken by some to indicate a shift of phosphorus from blood to tissues where it may be held in combination with the sugar which simultaneously disappears from the blood. This suggestion, however, has not been borne out by the work of Kay and Robison,[4] and Cori and Goltz[6] who were unable to find an increase, respectively, in muscle or in liver organic phosphates after insulin. Total blood phosphorus determinations, which might help to throw some light on this problem, have not been reported.

Since there appear, then, to be no clear-cut results indicating the fate of the phosphorus which seems to disappear after insulin administration, and since so little work has been done on the changes in all of the phosphorus fractions under the influence of insulin, further study of these problems is indicated.

This experiment was undertaken in order to observe changes in the sugar, inorganic phosphorus, total acid soluble phosphorus, lipoid phosphorus, and total phosphorus of the whole blood of rats on 3 different diets when insulin was administered to one-half of the animals 30 minutes before blood samples were taken. In none of the previous studies has any attention been paid to the habitual diet of the subjects, although it is well known that both blood sugar and blood phosphorus levels of animals in the post-absorptive condition may be affected by the composition of the diet.

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[1] Wigglesworth, V. B., Woodrow, C. F., Smith, W., and Winters, L. B., *J. Physiol.*, 1922-23, **57**, 447.

[2] Briggs, A. P., Koechig, I., Doisy, E. A., and Weber, C. J., *J. Biol. Chem.*, 1923, **58**, 721.

[3] Harrop, G. A., and Benedict, E. M., *J. Biol. Chem.*, 1924, **59**, 683.

[4] Kay, H. D., and Robison, R., *Biochem. J.*, 1924, **18**, 1142.

[5] Kerr, S. E., *J. Biol. Chem.*, 1928, **78**, 35.

[6] Cori, C. F., and Goltz, H. L., *Am. J. Physiol.*, 1925, **72**, 256.

The 3 diets, designated respectively as standard, high carbohydrate, and high fat, were planned to contain, as nearly as possible, comparable amounts of agar, cottonseed oil, protein, and salt mixture per calorie, as shown in Table I. In each case the diets were supplemented with 5.5 gm. of dried brewers yeast and 12 drops of codliver oil per animal per week.

TABLE I.
Composition of Basal Diets.

| Diet | Constituents % by weight | | % of Calories | Nitrogen % | Phosphorus % |
|---|---|---|---|---|---|
| Standard | | | | 2.6 | 0.38 |
| | Casein | 20.0 | 17.9 | | |
| | Cornstarch | 57.9 | 51.9 | | |
| | Hydrogenated Cottonseed oil | 14.0 | 28.3 | | |
| | Cottonseed oil | 0.9 | 1.8 | | |
| | Salts[13] | 3.6 | | | |
| | Agar | 3.6 | | | |
| High fat | | | | 3.7 | 0.54 |
| | Casein | 29.0 | 18.6 | | |
| | Cornstarch | 8.0 | 5.1 | | |
| | Hydrogenated cottonseed oil | 51.6 | 74.5 | | |
| | Cottonseed oil | 1.2 | 1.7 | | |
| | Salts[13] | 5.1 | | | |
| | Agar | 5.1 | | | |
| High carbohydrate | | | | 2.2 | 0.30 |
| | Casein | 17.0 | 17.9 | | |
| | Cornstarch | 76.0 | 80.2 | | |
| | Cottonseed oil | 0.8 | 1.9 | | |
| | Salts[13] | 3.1 | | | |
| | Agar | 3.1 | | | |

[13] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, **37**, 557.

Sixteen animals, taken when 28 days of age, were placed on each diet for 28 days. At the end of the experimental period, after 24 hours' fasting (except that in the first series those on the standard diet were fasted only 4 hours), one-half of each group received 3U/kg. of insulin and the others, acting as controls, were given no insulin. The pooled whole blood (collected under amytal anesthesia) from the 8 rats in each division was used to determine inorganic phosphorus by the method of Fiske and Subbarow,[7] total and total acid soluble phosphorus by a modification of the same method, lipoid phosphorus by a modification of the Bloor[8] and Fiske and Subbarow methods, and the blood sugar by Somogyi's modification of the Shaffer-Hartmann method.[9] The entire procedure was repeated with a second set of rats.

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[7] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

TABLE II.
Growth of Rats from 28th to 56th Day of Age on the 3 Diets.

| Diets | Series | No. of rats | Initial wt. gm. | Total gains gm. | Total food intake gm. | Food intake per gm. gain, gm. | Calories per gm. gain |
|---|---|---|---|---|---|---|---|
| Standard | 1 | 16 | 58 | 106 | 274 | 2.6 | 11.1 |
| | 2 | 16 | 62 | 140 | 330 | 2.4 | |
| High fat | 1 | 16 | 52 | 92 | 176 | 1.9 | 11.5 |
| | 2 | 16 | 61 | 106 | 190 | 1.8 | |
| High carbohydrate | 1 | 16 | 57 | 103 | 316 | 3.0 | 11.7 |
| | 2 | 16 | 62 | 101 | 316 | 3.1 | |

As can be seen in Table II, the food intake per gram gain in weight varies with the caloric content of the diet. Thus the animals ate almost exactly the same number of calories for each gram of gain in weight, in accord with the statement of Smith and Carey[10] that, within narrow limits, consumption of food by the rat is adjusted to caloric value of the ration. Moreover the amount of gain on all the diets was quite similar. These results, therefore, support the conclusions of Levine and Smith[11] that fat calories are as efficient as carbohydrate calories for the promotion of normal growth in the rat.

The results of the blood analysis are given in Table III and interestingly enough, in this experiment, the drop in inorganic blood phosphorus does not characteristically follow insulin injection but occurs only on the high fat diet, although it is found on this diet in both series. Most of the literature reporting changes in inorganic phosphate has dealt with inorganic phosphorus curves and not with a single point of phosphorus level after insulin. We arbitrarily chose one-half hour after administration of insulin as the time for taking the blood samples, since Barbour, Chaikoff, MacLeod, and Orr[12] have shown that there is a marked lowering of blood sugar within this period in the rat, and we have also found this to be the case. It is possible, however that at this time the inorganic phosphate had not yet approached its lowest level. If, in our study, within one-half hour after insulin injection, the inorganic blood phosphorus was just starting on the downward slope (and in all but one case, where there was no change, the trend after insulin was slightly in favor of a decrease) it would seem that a high fat diet increases the sensitivity of the inorganic phosphorus to insulin activity.

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[8] Bloor, W. R., *J. Biol. Chem.*, 1918, **36**, 33.

[9] Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599; 1930, **86**, 655.

[10] Smith, A. H., and Carey, E., *J. Biol. Chem.*, 1923, **58**, 425.

[11] Levine, H., and Smith, A. H., *J. Biol. Chem.*, 1927, **72**, 223.

[12] Barbour, A. D., Chaikoff, I. L., MacLeod, J. J. R., and Orr, M. D., *Am. J. Physiol.*, 1927, **80**, 243.

TABLE III.

Blood Sugar and Blood Phosphorus Partition in mg. % on the 3 Diets*

| | | Standard Diet | | High Fat Diet | | High Carbohydrate Diet | |
|---|---|---|---|---|---|---|---|
| Blood Constituent | Series | No Insulin | Insulin | No Insulin | Insulin | No Insulin | Insulin |
| Sugar | 1 | 139 | 38 | 99 | 37 | 83 | 24 |
| | 2 | 79 | lost | 88 | 21 | 74 | — |
| Inorganic Phosphorus | 1 | 6.3 | 6.0 | 6.1 | 4.3 | 6.0 | 5.9 |
| | 2 | 6.6 | 6.6 | 6.0 | 5.4 | 6.8 | 6.7 |
| Total Acid Soluble Phosphorus | 1 | 37.8 | 39.2 | 40.7 | 38.8 | 41.5 | 42.7 |
| | 2 | 39.9 | 41.3 | 40.7 | 41.3 | 42.8 | 43.9 |
| Lipoid Phosphorus | 1 | 12.7 | 11.3 | 11.5 | 10.3 | 10.3 | 11.3 |
| | 2 | 10.7 | 10.3 | 11.2 | 10.1 | 10.7 | 11.2 |
| Total Phosphorus | 1 | 51.5 | 50.9 | 51.8 | 50.4 | 52.4 | 53.8 |
| | 2 | 50.8 | 51.9 | 52.4 | 51.7 | 53.8 | 55.4 |
| Difference of Total Acid Soluble and Inorganic Phosphorus (Calculated Organic Phosphorus) | 1 | 31.5 | 33.2 | 34.6 | 34.5 | 35.5 | 36.8 |
| | 2 | 33.3 | 34.7 | 34.7 | 35.9 | 36.0 | 37.2 |

*Each figure represents the results of 2 to 4 duplicate determinations on the pooled whole bloods of 4 male and 4 female rats.

Since this is apparently the first attempt to observe changes in the inorganic blood phosphorus after insulin in the rat, the effect noted may be explicable as due to species differences in this matter between the rat and the rabbit, dog, and human subjects hitherto studied.

The total acid soluble and lipoid fraction are fairly constant on the 3 diets with and without insulin, slight changes in one partition being balanced by compensatory changes in another, so that the total phosphorus remains practically unchanged. The high carbohydrate diet is an exception, since there is some evidence of an increase in the total phosphorus on this diet following insulin administration. In any case, however, there does not appear to be a loss of phosphorus from the blood as suspected by other workers.

The calculated organic phosphorus figures are interesting in view of the controversy in the literature concerning the effect of insulin on this fraction. The changes that we found, an increase in the organic partition of about 3% in over 80% of the cases following insulin injection, are not of sufficient magnitude to warrant definite conclusions, but appear to indicate that there is a tendency, under the conditions of this experiment, for the blood organic phosphorus to increase during insulin activity. Such a change is in contrast with the results of Kerr[5] but in agreement with those of Briggs, Koechig, Doisy, and Weber[2] and those of Kay and Robison.[4]

*Conclusions.* It may be concluded, then, that after insulin injection, (a) the blood inorganic phosphorus of rats decreases only on the high fat diet, (b) the other determined phosphorus fractions vary only slightly, (c) there does not appear to be a loss of phosphorus from the blood, and (d) there is a tendency for the organic phosphorus of the blood to increase.

## 8210 C

## Intermediate Hosts of Aelurostrongylus Abstrusus of the Cat.

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The conception of the life history of the lungworm *Aelurostrongylus abstrusus* of the cat is based upon the work of Cameron as set forth in a series of papers by this author. No information, however, in regard to the development of intermediate forms of the first stage larvae in the supposed intermediate host, the mouse, is available from his studies.

Necropsies of 500 housecats of the city of San Francisco revealed the presence of *Aelurostrongylus abstrusus* in 8 instances. Twenty-two autopsies performed on cats from 20 other localities in California added 2 more cases. This material has been used in the present study.

The first stage larvae of *Ael. abstr.* behave in a manner usual among larvae of Synthetocaulinae known at the present time, according to our observations. Fry and Stewart[1] experimenting with these larvae state: "The larvae in this instance lived 18 days, which is opposed to Cameron's opinion that the larvae can not live longer than a fortnight in the free state." These authors believe that they may exist even longer under favorable conditions. In our experiments larvae have been found alive after a stay of 5 weeks in water. At this time we had to use them for other purposes. Water was chosen as a medium by reason of convenience. Under natural conditions the larvae may thrive on moist ground. According to Cameron: "The larvae of this species are carried to the exterior in the droppings, ingested by mice, continue their development and become encysted in these animals, and ultimately, when the rodent is eaten by a cat develop into the adult lungworms." (p. 58.)[2]

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[1] Fry, W., and Stewart, T. Th., *Parasitology*, 1932, **18**, 34.

"The faeces are eaten by mice and the larva migrates to various situations among the muscles and in the subcutaneous tissue, where, within 3 weeks it assumes an infective encysted form." (p. 66.)[3]

Cameron bases these conclusions upon his experimental work. One of his experiments—apparently his chief experiment—may be cited (pp. 102-103)[4] (pp. 59-60).[3] Forty-six mice were infected artificially. Eighteen mice died spontaneously owing to outbreaks of sarcosporidiosis and of rat bite fever. Eleven mice later showed cysts containing extra-intestinal larval nematodes. Six mice were negative as shown by investigation. Eleven mice were preserved for further investigation. However, Cameron did not find stages of development linking the larvae fed to the experimental animals to those observed in the 11 mice or in any of the 46 mice.

In 4 series of experiments undertaken by the writers in the course of the last 2 years to produce evidence of the development of the larvae in the mouse the result was negative. The experiment in one group may be briefly recorded. Twenty-four mice were fed with the first stage larvae of *Ael. abstr.* They subsequently were killed in groups of 2, after 1, 2, 3, 7, 14, 21, and 28 days. At no time, however, were larvae detected outside the intestine. The remaining 10 mice were destroyed 40 days after the beginning of the experiment. No larvae or cysts containing larvae were recovered from these mice. Thereafter they were fed to 2 kittens. Ten weeks later the kittens were autopsied. In neither of the animals could lesions of the lungs, embryos, eggs or adults of the lungworm be detected.

Recently Baudet[5] repeated Cameron's experiments of infecting mice with the larvae of *Ael. abstr.* with the same negative results; he knew of no reasons for this negative outcome.

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[2] Cameron, Th. W. M., *J. Helminth.*, 1926, **4**, 53.

[3] Cameron, Th. W. M., *J. Helminth.*, 1927, **5**, 55.

[4] Cameron, Th. W. M., *Vet. J.*, 1928, **85**, 97.

[5] Baudet, E. A. R. F., *Tijdschr. v. Diergeneeskunde*, 1933, **60**, Afl. 18, 1.

Consequently an inquiry was undertaken by the writers to determine the nature of the apparently still unknown intermediate host of the lungworm.

We used in our work larvae either taken directly from the lungs or those isolated from the faeces of affected cats. We, however, were not able to raise the first stage larva itself from the unsegmented egg of the parasite as Cameron was able to do. He reiterates with emphasis this fact—to have raised the larvae outside the vertebrate host in the body of female worms of *Ael. abstr.* and corroborates this statement with camera lucida drawings (p. 57).[3] Cameron makes no mention, however, of the technique applied to secure these results, which, successfully attained, would be extraordinary in any species of Synthetocaulinae.

Several fruitless attempts were made by the writers to infect small vertebrates and certain insects, which occasionally might be taken by cats as food. Positive results were obtained finally by the introduction of different mollusks. Snails of the genus *Epiphragmophora Doering,* 1873, proved to be the most suitable intermediate hosts. *Helminthoglypta* (E.) *californiensis,* Lea; *Helminthoglypta* (E.) *nickliniana,* Lea; and *Helminthoglypta* (E.) *arrosa,* Gld.* were infected successfully. Furthermore, third stage larvae could be raised in *Helix aspersa,* Mueller, in *Agriolimax agrestis,* Linné, and in *Ariolimax columbianus,* Gld. These observations show again that lungworm larvae of Synthetocaulinae are linked rather with a wide group of correlated intermediate hosts than with a single species. In a few of them, the larvae encounter optimal conditions and develop in considerable numbers; meanwhile, even after heavy infestations, in others only a restricted number of larvae result due to less favorable conditions. In the case of *Ael. abstr.,* Epiphragmorphora in its different species is the most favorable intermediate host in California. In the other snails and especially in the slugs named above the final number of third stage larvae remained restricted, the time for their development varied, and their life span appeared shortened.

The invasion of the mollusks takes place in the manner previously described in other Synthetocaulinae.[6] Shortly after invasion, movements of the larvae decrease and they finally coil up. Their length increases to a less extent than their width. Fat and protein granules develop and conceal their inner structure. The first molt occurs about 10 days after infection. The larva now appears stout and its body becomes more delicate. The cuticle loosens from the body first in the head and tail regions. Finally it may surround the second stage larva as a wide sheath (Fig. 1). The cuticular parts of the entrance of the esophagus, of the excretory pore, and of the cuticular rectum of the first stage larva remain attached to the sheath as ordinarily. No ecdysis follows. The enclosed second stage larva measures 380 to 400μ in length by 36 to 38μ in width just below the esophageal junction. It attenuates toward both ends, more, however, toward the head region than toward the tail. The newly formed tail is short and it ends straight and sharp pointed. The undulating sigma form and the dorsal spine of the first stage larva are absent on it. The tail is only 20μ long. The esophagus is more slender and it measures about 160μ in length. It shows only one slight bulb at its junction with the intestine. With the approaching second molt the accumulated fat and protein granula begin to dissolve. This process proceeds from the esophageal region slowly toward the posterior parts of the larvae.

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* We are indebted to Dr. G. D. Hanna, of the Academy of Sciences, San Francisco, who assisted us in the classification of the snails.

[6] Hobmaier, M., *Z. f. Parasitologie,* 1934, **6**, 642.

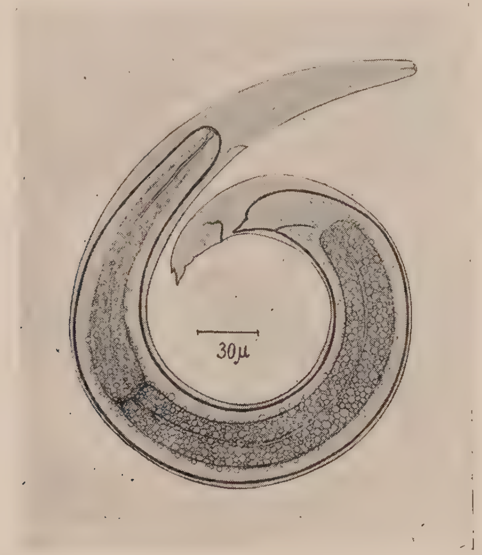

FIG. 1.
*Aelurostrongylus abstrusus.* Second stage larva from *Helminthoglypta* (E.) *arrosa* Gld.

Four to 5 weeks or even later after the beginning of the experiment the second and final molt of the larva in the mollusk may be observed. The reasons for this variation in time are not definitely known. Chief factors may be the species of intermediate hosts involved, the varying localization of the larvae in the mollusks and above all certain seasonal factors. The newly formed larva, representing the third or infestive stage, is slender in appearance compared with the second stage larva (Fig. 2). Nothing is known at the present time about the metabolism of the larva during its stay in the mollusk. The accumulation of steadily increasing quantities of fat and protein granules beginning with the time of the immigration of the larva and ending with its first molt seems to indicate that fluids of the host tissues are used as foodstuff during this period. Meanwhile the formation of the final third stage larva may be accomplished by the exclusive use of these reserves. The second phase of the intramolluscan life of the young nematode is characterized by the transformation of the reserves into well defined cellular structures. After the second molt has occurred the dissolution of the fat and protein granules progresses until the body of the larva appears clear in its lines. The stay of the third stage larva may extend over months, but otherwise no particular changes are to be observed during this time.

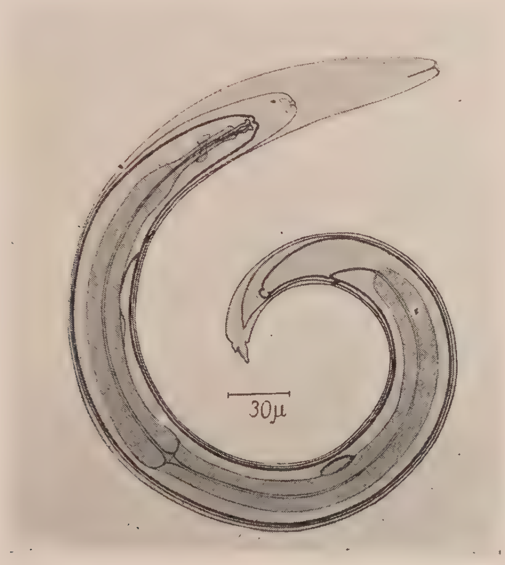

FIG. 2.
*Aelurostrongylus abstrusus.* Third stage larva from *Helminthoglypta* (E.) *arrosa* Gld.

The larva enclosed in its 2 sheaths measures, according to its state of contraction, 450 to 520μ in length by 26 to 28μ in width, after a stay of about 6 weeks in its intermediate host. The length may increase a little during the following months. Both ends of its body are slightly attenuated. The mouth opening is simple. The esophagus, measuring 200 to 225μ in length has now a more muscular appearance. It is still composed of 3 parts but has only one slight bulb at its junction with the intestine. The excretory pore opens 90μ behind the head of the larva. The intestine is colorless. It, however, later may show a slightly yellowish brown color. It forms a small tube with a diameter of about 18μ. It ends in a small cuticular rectum 26 to 30μ in length. The primordial cells are situated near the middle of the intestine. The anus ends 30μ in front of the tip of the tail. Immediately behind the anus the larva attenuates to a short stout tail with a little flat knob on its end. It carries no papillae. After artificial liberation of the larva from its sheaths the knob stretches to the extent that the little appendix may present itself in the shape of a short finger. The larva is now more resistant toward injury possibly caused by microscopic investigation than previously. Slight pressure releases the larva from its sheaths. It moves forth and back for some time in the surrounding tissues; finally, however, it coils up, but death may be delayed for a few days. Larvae have been kept in mollusks under observation for a period of 6 months. At the end of this time little change had taken place in larvae located in Epiphragmophora. In *Helix aspersa* they appeared enclosed in stronger tubercle formations surrounded by yellowish caseous material. The larvae themselves were feeble but most of them still were alive. In the other mollusks mentioned above the greater number of larvae had disappeared already or were found to be lifeless.

Third stage larvae raised in the different species of Epiphragmophora and of *Helix aspersa* as intermediate hosts were fed separately to uninfected cats and kittens. The different species of slugs, however, were fed simultaneously to the animals. Adult lungworms of *Ael. abstr.* were recovered from the experimental animals in all cases by autopsies. An account of the development of the adult stages and of the possible rôle of mice in the life cycle of the parasite will be presented in subsequent papers.

*Conclusion.* The occurrence of the lungworm *Aelurostrongylus abstrusus* of the cat in California was established. About 2% of the cats investigated were found infected with the parasite. Different species and varieties of Epiphragmophora were found serving as principal intermediate hosts. Other snails and slugs mentioned above seem to be of minor importance. The development of the third stage larvae is described and subsequent infections of cats and kittens are recorded. Mice fed with first stage larvae of the lungworm escaped infections. The intermediate hosts of the lungworm *Aelurostrongylus abstrusus* are not, as Cameron states, mice, but certain mollusks.

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### 8311 P

## Route of Transmission of St. Louis Encephalitis Virus in Mice.*

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Histopathological studies by Webster and Fite[1] suggest that the virus isolated from the St. Louis outbreak of encephalitis, travels by way of the central nervous system in mice. The present report confirms and extends these findings.

In order to determine the portal by which mice could be infected, they were given virus by the nasal and gastro-intestinal routes. Three series of mice each consisting of 4 to 6 animals, were given a concentrated suspension of the virus in their food over 2 to 3 days. Not one of these mice became infected. Three animals, upon whom a laparotomy was performed, were injected intrastomachally with 0.2 cc. of a thick virus suspension. None of these showed evidence of the disease.

Mice could be infected regularly with the intracerebral virus, within an incubation period of 6-8 days, by placing 0.03 cc. of a 1% suspension into one nostril, while 0.1% failed to give regular infection. The virus was transmitted serially by the intranasal route for 28 passages, using a 10% suspension, but its infectivity, by this route, did not increase.

After 28 passages the virus was infective by the intranasal route, at approximately a 1:250 dilution.

In order to trace the route of infection from the nasopharynx,

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*This research was aided by grants from New York and Rockefeller Foundations.

[1] Webster, L. T., and Fite, S. L., *J. Immun.*, 1934, **26**, 344; *Am. J. Path.*, 1934, **10**, 666.

the blood, parenchymatous tissues and various areas of the cerebrospinal axis were tested for the presence of virus after intranasal instillation of virus at various stages of the incubation period and of the disease. Two experiments were carried on simultaneously; one in which various organs and areas of the cerebrospinal axis were suspended in distilled water and one in which 10% normal monkey serum, which enhances the infectivity of the virus, was employed as diluent. In testing any area, a suspension made of a pooling of 2 to 3 organs in that area was injected intracerebrally and intraperitoneally Control animals receiving intranasal inoculations of the same virus were included in east test and always showed symptoms.

On only 2 occasions at 3 hours and 48 hours was virus demonstrated in the blood. It was demonstrable in the parenchymatous organs only occasionally and then usually early or late in the disease. On the other hand, it was present in the olfactory bulbs as early as 2 hours after inoculation. The next location of the virus was the lobus pyriformis, while late in the incubation period or early in the disease, it had spread throughout the whole central nervous system.

The findings indicate that the virus enters by the olfactory rather than the gastrointestinal tract. Moreover, inasmuch as the virus is rarely found in the blood stream, as indicated by these experiments and those of Webster and Fite,[1] and since we were unable to infect regularly by the skin route with less than 0.5 cc. of a 10% suspension, insect transmission is unlikely. The virus then appears to travel along the nerve fibers of the olfactory nerve to the bulb and thence to the lobus pyriformis, after which it spreads to all parts of the central nervous system. These experiments confirm the histopathological findings of Webster and Fite, who found that the lesions appeared in the above mentioned sequence after intranasal instillation of virus. It is interesting to note that the virus appears earlier than do the lesions, for these authors did not find lesions in the olfactory bulbs until the third day after intranasal inoculation, whereas they found virus present 2 days after nasal instillation and in the present work it has been reported as early as 2 hours after the introduction of the virus. Likewise, the virus is present in the lobus pyriformis prior to the presence of cellular infiltration, indicating that the lesions are the result of damage by the virus.

The early, occasional demonstrable virus in the parenchymatous organs or blood stream may be due to absorption by the nasal mucosa. Its more frequent later appearance may be due to an over-flow of the virus from the perivascular spaces of the central nervous system, into the blood and thence to the organs.

### 8212 P

## "Mottling of Enamel" Effected by a Single Fluorine Dose.

S. LOEWE AND H. SALFELD. (Introduced by H. Sobotka.)
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In a study of the minimal effective dosage of fluorine, as determined by various biological criteria, one of the authors[1] observed "fluorine-rickets" as a significant response to very small amounts of fluorine. The biological effectiveness of even smaller amounts of this element has been established by the discovery of the "mottling of enamel."[2] Until now, mottling of enamel has been studied only on frequently repeated fluorine administration. The question arose whether it can also be produced by a short "fluorine shock."

The following is a report on a preliminary series of experiments on 20 young rats. In the first experiment (Table I) doses from 9 to 37 mg. fluorine per kilo of b.w. were administered through a stomach tube to just weaned albino rats weighing from 21 to 35 gm. (solutions of 0.625% or less NaF and a finely dispersed sol of 0.375% or less $CaF_2$[3]). Such a single dose was able to produce a definite alteration on the enamel. In 3 out of 10 animals, a sharply

TABLE I.
Effect of Fluorine Administration on Incisors of Recently Weaned Rats.

| No. | Wt at start gm. | Wt on 11th day gm. | Administered mg. F per kg. b. w. | Enamel Spot Day of Appearance | Enamel Spot Day of Disappearance |
|---|---|---|---|---|---|
| 1 | 31 | 25 | $CaF_2$ 37 | — | — |
| 2 | 29 | 30 | NaF 30 | — | — |
| 3 | 23 | 32 | $CaF_2$ 19 | — | — |
| 4 | 23 | 34 | $CaF_2$ 19 | — | — |
| 5 | 35 | 38 | NaF 17 | 12 | 24 |
| 6 | 30 | 38 | NaF 15 | — | — |
| 7 | 36 | 39 | $CaF_2$ 10 | — | — |
| 8 | 28 | 29 | $CaF_2$ 9 | 9 | 21 |
| 9 | 35 | 36 | $CaF_2$ 9 | 9 | 24 |
| 10 | 31 | 37 | NaF 7 | — | — |

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[1] Loewe, S., *Schweizer med. Ws.*, 1934, **64**, 1177.

[2] Smith, M. C., and Leverton, R. M., *Ind. Eng. Chem.*, 1934, **26**, 792; De Eds, F., *Medicine*, 1933, **12**, 1.

[3] Bachmann, W., D. R. P., 485,052.

limited milky white spot of about 1-2 mm. in diameter, round or oval, appeared on the frontal surface of one lower incisor between the 12th and 14th day following fluorine ingestion. First, the spot was seen only at the base of the incisor after slight retraction of the gum. Later, it moved slowly along the tooth and finally between the 21st and 24th day it disappeared at the cutting surface. The incidence of positive results is not increased by increasing the fluorine dosage, nor does it depend on the degree of general effects of fluorine shown by the growth curve (*cf.*, animal No. 1 vs. animal No. 6). Nor is there an obvious difference between the 2 compounds used. Thus, fluorine is but one of several factors capable of impairing enamel development. In the range of the dosage studied the behavior of these other coefficients (*e. g.*, vitamin D, food calcium, food fluorine, natural irradiation) must be decisive for the issue.

In another group, single doses of 20 or 30 mg. F per kg. as NaF and 10 or 30 mg. F per kg. as $CaF_2$ were administered to 10 albino rats of 40 to 116 gm. No lesions were detectable; thus, age is a further factor controlling fluorine action on enamel formation.

None of the coefficients mentioned above deviated sufficiently from its physiological range as to produce mottling of enamel by itself without fluorine administration. A careful observation of 40 control animals from the age of weaning showed no spontaneous mottling.

In spite of the irregularity of the results, the experiments demonstrate that a short "fluorine shock" is sufficient to evoke irreversible changes in enamel, presumably by inhibiting a phosphatase action necessary for the process of calcification. The effects of intermittent subcutaneous injections of fluorine on rats, recently reported by M. C. Smith,[4] point in the same direction: Stripes of defective calcification alternating with normal enamel areas are produced by this interrupted chronic treatment. This fluorine shock permits one to mark by an indelible stamp on the tooth surface the date of fluorine administration.

Acute and chronic fluorine action differ only in the extensiveness of the effect and in the effectiveness of the dosage. Single administration causes a single lesion, repeated administration produces mottling. The minimal single dose that may possibly produce an effect is yet to be determined, the very low daily dose invariably effective in chronic experiments fails to produce macroscopically detectable injuries, if administered only once. The larger single dose necessary to produce enamel injury, also causes general effects. The high selectivity (*i. e.,* the absence of other detectable symptoms) of the enamel mottling action in chronic fluorine administration is not encountered in acute experiments.

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[4] Smith, M. C., *A. J. P. H.*, 1935, **25**, 698.

## 8213 P

## Histological Changes Observed in the Intestinal Wall Following Simple Mechanical Obstruction in Rabbits.

MIMS GAGE AND KIYOSHI HOSOI.

*From the Department of Surgery, Tulane University School of Medicine, New Orleans, La.*

Due to the almost miraculous recovery of the intestinal wall in cases of ileus after surgical relief of mechanical obstruction as well as the recovery of the intestinal wall in certain forms of mesenteric thrombosis after simple enterostomy, this investigation was undertaken to demonstrate what changes, if any, may occur following simple mechanical obstruction in the various tissues of the intestinal wall.

Simple mechanical ileus of the terminal ileum was produced in 11 rabbits. At varying postoperative intervals (12, 24, 36, 48, and 72 hours), the rabbits were sacrificed, and sections from 3 different levels of the ileum were removed—1 cm., 10 cm., and 20 cm. proximal to the obstructing ligature. Sufficient intestine was removed from each level to obtain 10 specimens for immediate fixation, making a total of 30 gross sections from each animal. For controls, sections were removed also from a normal rabbit corresponding to the different levels mentioned above.

Gross lesions observed postoperatively were: After 12 hours, ileum proximal to the obstruction was reddish in color and on section some blood was seen in the lumen. 24 hours: Slight distention of the terminal ileum; lumen filled with liquid intestinal contents; very few gross changes were evident. 36 hours: Distention of the terminal ileum; few ecchymotic spots in the mucous membrane; intestines filled with liquid feces. 48 hours: Marked distention of ileum with considerable congestion; contents yellow watery fluid; ecchymotic areas in mucosa near the obstruction. 72 hours: Marked distention, especially just above the obstruction, and containing much yellowish-red fluid, with marked congestion of the ileum and minute mucosal hemorrhages. Thrombosis of mesenteric vessels were never observed.

Staining methods used were: Hematoxylin and eosin, Gram's stain, Verhoeff's stain, Mallory's phosphotungstic acid hematoxylin, Laidlaw's lithium silver stain, Foot-Mènard's ammonium silver stain, Müller's osmic acid stain, Masson's trichrome, Ramon y Cajal's Formula 3a and Formula 6a. In comparison with control sections of the normal rabbit, the mucosa and muscularis after obstruction showed no essential changes. It is remarkable that even after 72 hours of obstruction, the mucosal lining cells showed no noticeable degenerative changes. The nuclear elements stained with great clearness.

Blood vessels showed no appreciable changes in the sections removed after 12 and 24 hours of obstruction. After 36 hours, in the soft tissues of the submucosa there was an increase in number and some distention of blood vessels. In one 36-hour specimen, a small submucosal hemorrhage was observed. By the 48th hour, all the vessels of the mucosa and submucosa were markedly engorged with surrounding minute hemorrhages. The lymphatic channels of the villi first became noticeable as oval and cylindrical dilated spaces. In proportion to the duration of obstruction, the dilatation of the lymph channels increased, reaching its maximum distention after 36 hours of obstruction. Following this maximum distention of central main lymph vessels, the adjacent tissue spaces became quite edematous, so that the stroma of the villi appeared as a lacy network. However, the edema of the submucosa was first noticed in the 12-hour obstruction, and progressively increased in intensity.

In the 12-hour sections, the intestinal wall near the site of the obstruction was diffusely but moderately infiltrated with polymorphonuclear leucocytes. At the 10 and 20 cm. levels, the leucocytic infiltration was still present but progressively less. After 24, 36, 48, and 72 hours, the leucocytic infiltrations were not as marked as after the 12-hour obstruction, being least evident in 72-hour specimens. This indicates that the leucocytic response was due to the operative trauma and not to the obstruction *per se.*

As regards the connective tissue derivatives (fibroglia, elastic fibers, and reticulum), no abnormalities could be seen. Few or no changes were observed in the nervous elements of the intestinal wall. Many of the ganglion cells of Auerbach and Meissner continued to exhibit well preserved Nissl bodies even after 72-hour obstructions. The neurofibrils of these nerve cells and their terminal finer arborizations were all well stained.

In the normal sections stained after Gram's method, only an occasional coccus or diplococcus could be found as gram-negative organisms in monocytes. After the production of obstruction, detailed examination of the endothelial lining cells of the distended lymph channels under oil immersion showed noticeable numbers of phagocyted gram-negative cocci, mostly diplococci. It was only after 48-hour obstructions that definitely gram-positive cocci were observed. The bacteria were first observed in the villi; after 24 hours of obstruction, bacteria began to be found in the submucosa, first as isolated diplococci and finally as definitely noticeable clumps and more numerous at about the 48-hour obstruction level. We were not able to find gram-positive or negative bacilli within the intestinal tissues. Most of the cocci were gram-negative and all phagocyted by the monocytic endothelial cells, indicative of the remarkable protective power of these cells in spite of the obstruction persisting for as long as 72 hours.

After 72 hours, the changes in the intestinal wall must have occurred with great rapidity involving all the tissues, as no animal survived up to 96 hours. Four rabbits were used; all died before 96 hours and marked postmortem changes were found, prohibiting any histological study.

## 8214 P

## Effect of Multiple Pituitary Primordia in the Tadpole.*

WILLIAM ETKIN. (Introduced by G. K. Noble.)

*From the College of the City of New York, and American Museum of Natural History.*

That the pituitary plays a prominent part in controlling metamorphosis and sexual maturity in vertebrate development is clearly established. But little is known of the factors that determine pituitary activity at these times. This study is directed at the analysis of these factors in the metamorphosis of the tadpole.

The experimental animals drawn from the same batch of *Rana sylvatica* eggs were prepared as follows: (1) 30 normal unoperated controls; (2) 116 in which the buccal primordium of the hypophysis was removed in the tail bud stage; (3) 48 hypophysectomized, but with the hypophyseal primordium reimplanted either into the eye cup or under the adhesive discs; (4) 50 as in No. 3, but with

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* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

the addition of 3 primordia implanted under the adhesive discs. The superficial ectoderm was generally included with a minimum of brain tissue, but no attempt was made to remove all possible adherent brain. When the animals were sufficiently developed to determine the success of the grafts in terms of the pigmentation reaction they were made up into sets consisting of one "successful" animal of each type. Of 10 sets 6 were carried through metamorphosis successfully. The animals were kept at 26-29°C.

Group 4 with 4 pituitary transplants metamorphosed precociously as compared to groups 1 and 3. The time required to accomplish a definite metamorphic change, the resorption of the anal canal piece, in group 4 averaged 7.8 ± 1.4 days after the experiment was set up. For groups 1 and 3 these figures were 17.7 ± 0.6 and 19.8 ± 1.7 days respectively. A more significant measure of precocity is the maximal size attained by the tadpoles previous to the reductions incident to metamorphosis. This averaged 28.7 ± 1.5 mm. for group 4 and 43.5 ± 0.6 mm. and 45.2 ± 1.0 mm. for groups 1 and 3. The group 4 animals were not only smaller than their controls at metamorphosis but in 5 of the 6 cases entirely below the range of variation of normal animals as seen in the author's experience with this species. The animals with single grafts were not accelerated. There was possibly retardation and greater variability than the controls. The hypophysectomized animals of group 2 showed no metamorphosis. Increased pigmentation occurred in some animals of groups 3 and 4. But such pigmentation was not conspicuous nor constant, and was independent of metamorphic precocity.

Serial sections of 3 heads of group 4 animals revealed 2 to 5 pituitary fragments in each. Three of these fragments were entirely out of contact with either brain or retina, and 3 were in contact with the retina but not with the brain proper. The differentiation of all grafts appeared more nearly like that in tadpoles of similar age than of similar metamorphic stage.

These results differ from Blount's[1] experiments with Amblystoma in the following points: He found (1) no precocious metamorphosis; (2) distortion of body proportions; (3) no differentiation of epithelial hypophysis independent of the infundibulum; (4) intensified pigmentation invariably concomitant with successful multiple pituitaries.

The significance of my experiment appears to be as follows: Since the multiple pituitaries were potent for thyroid activation in the body of an immature host the pituitary appears to be physiologically self-differentiating and capable of thyrotropic activity independently of specific nervous or hormonal stimulation from any other gland in the body. Further the concept is suggested that the thyroid is dependent upon the quantity of pituitary and not on qualitative alteration of pituitary activity during development.

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[1] Blount, R. F., *J. Exp. Zool.*, 1932, **63**, 113; 1935, **70**, 131; *Anat. Rec.*, 1935, **61**, Suppl., 6.

## 8215 C

## Further Purification of Galactin,* The Lactogenic Hormone.

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Since the first reports of the extraction of the lactogenic hormone by Gardner and Turner,[1] and Riddle, Bates and Dykshorn[2] involving the isoelectric precipitation of the active material from either an alkaline or acid digest of fresh or desiccated ground anterior pituitaries of sheep, cattle or hogs, little progress in purification has been reported.

The present paper presents the details of a method of further purification by which approximately a ten-fold concentration of the active principle may be effected.

The isoelectric precipitate obtained over a range of pH of 6.5 down to 5.5 from the alkaline digest is centrifuged for 20 minutes and the filtrate decanted. The precipitate is then dehydrated by repeated treatment with acetone and dried in a desiccator. It is then ground into a fine powder.

Four grams of the acetone dried powder is then thoroughly mixed with twice its volume of glacial acetic acid, centrifuged, and the brown supernatant liquid decanted into 12 times its volume of ethyl ether. This glacial acetic acid treatment is repeated 4 times or until the brown color no longer appears in the supernatant liquid. The supernatant acid from each of the extractions is decanted into the preceding acid-ether mixture. Lengthy acid treatment is to be avoided. A white flocculent precipitate containing the active principle settles out when the extraction liquids are added to the ether. The acid-ether liquid is decanted, fresh acetone is added to the precipitate and the mixture allowed to stand for 45 to 60 minutes. After centrifuging the acetone is decanted and the precipitate placed in the desiccator. After it is dry it may be ground to a fine white powder. The yield is usually about 0.4 gm. or 10% of the original precipitate. In amounts tolerated, the glacial acetic acid insoluble fraction appears to be inactive.

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* Also called prolactin and mammatropin.

† Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 406.

[1] Gardner, W. U., and Turner, C. W., Mo. Agr. Exp. Sta. Res. Bul. 196, 1933.

[2] Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

The active extract gives a biuret test for protein. A total of 3 mg. administered to common pigeons weighing from 300 to 325 gm. over a period of 4 days in daily injections will cause the crop glands to increase from an average of 1.5 gm. to between 1.7 and 3.8 gm. (average 2.73 gm.) showing also very distinct evidence of the proliferation of the crop gland.

## 8216 P

## Cytology of Psittacosis Virus in the Sparrow (Zonotrichia).

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The great susceptibility of the representatives of the bird family Fringillidae for the psittacosis virus has previously[1] been emphasized. In the course of systematic studies relative to the various birds which might possibly serve as hosts for the virus, a series of white crowned *(Zonotrichia leucophrys gambeli)* and golden crowned sparrows *(Zonotrichia coronata)* were injected intraperitoneally with 0.3 to 0.5 cc. of a 10% organ suspension in broth of a mouse or Java bird—passage virus (mouse M.L.D. $10^{-8}$). Many of the injected sparrows, which as a rule died in from 8 to 12 days, revealed at autopsy a diffuse plastic exudate teeming with free and intracellular Levinthal-Cole-Lillie bodies. The exudate covered both the pericardium and abdominal viscera. Victoria blue 4 R. was particularly useful to demonstrate the free elementary bodies. The liver necroses and the splenic tumor so commonly observed in the representatives of the Psittacidae and some of the Java birds infected with the psittacosis virus were missing. The exudate proved highly infectious and served as a useful antigen for various serologic reactions. Moist and dry fixed impression preparations stained by the Giemsa method and differentiated with acetone or methyl alcohol disclosed the developmental stages of the virus. Bedson and Bland[2] have reported the virus cycles in a rodent (mouse) and Bland and Canti[3] have described the growth phases of the psittacosis infective agent in chick embryonic tissue cultures. But since thus far no detailed records are available concerning the behavior of the virus in birds, it seemed advisable to analyze the cytology of the sparrow infection.

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[1] Meyer, K. F., and Eddie, B., *Klin. Wchnschr.*, 1934, **13**, 865; *Berl. tierärztl. Wchnschr.*, 1934, **50**, 577; Proc. Soc. Exp. Biol. and Med., 1934, **31**, 917.

The young birds injected intraperitoneally were sacrificed at different intervals of time following the inoculations. Quantitative virus tests on mice accompanied the cytological studies on stained and unstained impression preparations of the exudate. The correlated findings on 25 different sparrow infections are as follows:

Although a casual examination of the preparations leaves little doubt that the development of the various virus colonies is entirely an intracellular cytoplasmic process, one encounters difficulties in demonstrating the early stages. In the exudate of birds sacrificed on the 5th day, a large number of monocytes are vacuolated and contain light or deep purple staining oval, circular or comma shaped bodies of varying sizes; in some cells these elements are arranged in irregular aggregates which are perhaps the result of coalescence of the purple bodies and thus resemble the "plaques" of Bedson and Bland. They vary in diameter from 2 to 12μ and are frequently located at the margins of the monocytes. A homogeneous matrix surrounded by a membrane or capsule sharply distinguishes these bodies from the cytoplasm of the host cell. In the Giemsa preparations the matrix stained either lightly or deeply and of a greenish or turquoise blue shade retains a few or numerous large intensely colored particles. Some of these cells with large granules resemble the Koch's blue bodies of African East Coast fever. When the peritoneal exudate is profuse and the infection of the mesothelial cells is heavy, the cytoplasm is quite often inhabited by multiple colonies in continuous stages of development. The membrane encapsulating these colonies may not be very distinct and quite often they fuse irregularly. Within the matrix, the large and small particles retain the dye with variegated degrees of intensity. The final evolution of these colonies to an aggregation of elementary bodies is particularly clearly demonstrable in moist fixed preparations (Schaudinn). Since the monocytes with the virus colonies exhibit changes due to injury, it is reasonable to understand that the disintegration or rupture liberates the elementary bodies which may appear as clusters or clumps or as free particles of relatively uniform size in enormous numbers throughout the exudate. In very resistant cells the colonies of elementary bodies may assume enormous size and fill the entire cytoplasm. Finally it is noteworthy that both free elementary bodies and their progenitors—the cytoplasmic colonies with the large particles—are observed at the time of death in the preparations made from the peritoneal exudate of the infected sparrows.

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[2] Bedson and Bland, *Brit. J. Exp. Path.*, 1932, **13**, 461; 1933, **14**, 267; 1934, **15**, 243.

[3] Bland and Canti, *J. Path. and Bact.*, 1935, **38**, 233.

### 8217 P

## Some Observations Upon Blood Glucose in Epilepsy.

VICTOR G. HAURY AND ARTHUR D. HIRSCHFELDER.*

*From the Department of Pharmacology, University of Minnesota.*

Pigott[1] has recently reported low blood sugar in epileptic convulsions and regards hypoglycemia as a possible etiological factor. In our series of 25 cases in which 3 or more determinations have been made in each case we do not confirm this view. The blood glucose was determined by the method of Gibson.[2] Determinations were made before, during and after seizures and in each case the blood glucose was within normal limits (90 to 110 mg. per 100 cc. blood) before convulsions, and rose during the convulsions. The rise of blood sugar was roughly proportional to the severity of the seizure, and after termination of the convulsion the blood sugar returned to normal in two to four hours.

In 4 cases of *status epilepticus* the blood sugar value before seizure was 95.0 to 100.0 mg. per 100 cc. blood. After about one hour of status the values rose to 139.0 to 190 mg. per 100 cc. and after three hours of status the values were 150 to 240 mg. per 100 cc.

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* Supported by a research grant from the Eli Lilly Co.

This investigation was conducted at the Minnesota Colony for Epileptics, whose clinical material and laboratory facilities were placed at our disposal by the Minnesota State Board of Control. To them, to Dr. D. E. McBroom, Superintendent of the Minnesota Colony for Epileptics, and to Dr. R. W. Brown we desire to express our thanks for their valuable cooperation.

[1] Pigott, A. W., Read before the Amer. Psychiatric Assoc., Washington, May 14, 1935.

[2] Gibson, R. B., Proc. Soc. Exp. Biol. and Med., 1930, **27**, 480.

In 21 cases having moderate to very severe isolated seizures the blood sugar rose from the average of 92.5 mg. per 100 cc. blood before seizures to 168.0 mg. per 100 cc. during convulsions. In 7 cases the elevation of blood sugar was above 185 mg. per 100 cc. during the height of the convulsions.

In no instance was hypoglycemia found either before or during the convulsions.

### 8218 P

## Synaptic Transmission in the Stellate Ganglion.*

D. W. BRONK, S. S. TOWER, AND D. Y. SOLANDT.

*From the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania.*

We have investigated certain aspects of the mechanism of synaptic transmission in a sympathetic ganglion by recording the action potentials in the inferior cardiac nerve of the cat while stimulating the preganglionic fibers to the stellate. This preparation has proved admirable for such a study because the long postganglionic nerve makes possible a careful investigation of the properties of the fibers whose response is used as a measure of the activity of the ganglion.

The form of the action potential in the non-medullated fibers of the inferior cardiac nerve has been determined by recording the impulses in the nerve during direct excitation. It differs from that of medullated nerve in 2 respects which are important to this investigation. Because the conduction velocity is much less (0.4 to 1.1 meters per second) there is a marked spread of the spike potential due to temporal dispersion of impulses in the several fibers. This temporal dispersion and the large positive and negative after potentials which are found in these non-medullated nerves may combine to give the potential wave a complicated form of several crests and troughs. In this we confirm Bishop.[1] Because of these considerations it is necessary to be cautious in the use of the post-

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* The expenses of this investigation were defrayed in part by a grant from the Committee on Grants-in-Aid of the National Research Council.

[1] Bishop, G. H., *J. Cell. and Comp. Physiol.*, 1934, **5**, 151.

ganglionic response as an index of repetitive firing from the ganglion or in the use of the ganglion potentials described by Eccles[2, 3] as a key to ganglionic processes.

The question as to whether each preganglionic volley sets up a single postganglionic volley or whether an independent rhythm is developed in the synapses of the ganglion has long been debated.[4, 5, 6] We have reopened the problem with the following experiments. The preganglionic nerves have been stimulated with single and repeated shocks and the potentials in the postganglionic nerves recorded. Provided the frequency of stimulation does not exceed about 40 per second we find that each volley of impulses in the preganglionic fibers sets up only a single volley of impulses in the postganglionic nerve.

But the ganglion introduces a certain amount of temporal dispersion into the volleys either because of differences in synaptic latency, or because of repetitive firing within the volley by the individual units, or because of both these factors. This follows from the observation that the spike potential in the postganglionic nerve is always more dispersed temporally when the impulses are transmitted through the ganglion than when merely conducted through an equal length of postganglionic nerve. The duration of this discharge from the ganglion is frequently as long as 50 milliseconds and may be longer as a result of previous stimulation. On the other hand, there is no evidence of after discharge in the form of successive, fairly definitely synchronized volleys set up by a single preganglionic volley.

We have frequently observed that the postganglionic response becomes progressively larger during the course of repetitive submaximal stimulation of the preganglionic nerve. This is due to the excitation of more and more postganglionic fibers by the same number of preganglionic fibers and is presumably related to the process of facilitation in the ganglion described by Eccles.[3] We have noticed this phenomenon with stimulation frequencies of about 5 per second, and the increase in response continued for over 10 seconds.

The work of Feldberg and Gaddum,[7] Feldberg and Vartianen[8] and others indicates that the postganglionic fibers are stimulated by

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[2] Eccles, J. C., *J. Physiol.*, 1933, **80**, 25 P.

[3] Eccles, J. C., *J. Physiol.*, 1934, **81**, 8 P.

[4] Querido, A., *Am. J. Physiol.*, 1924, **70**, 29.

[5] Bishop, G. H., and Heinbecker, P., *Am. J. Physiol.*, 1932, **100**, 519.

[6] Knoefel, P., and Davis, H., *Am. J. Physiol.*, 1933, **104**, 81.

[7] Feldberg, W., and Gaddum, J. H., *J. Physiol.*, 1934, **81**, 305.

[8] Feldberg, W., and Vartianen, A., *J. Physiol.*, 1934, **83**, 103.

acetylcholine liberated at the preganglionic endings. In support of that hypothesis they find that perfusion of the superior cervical ganglion with Locke's fluid containing eserine, which protects acetylcholine against cholinesterase, increases the contraction of the nictitating membrane elicited by a submaximal preganglionic stimulus. In order to test this theory of synaptic transmission by a different method we have injected eserine intravenously in amounts such that the concentration in the blood circulating through the ganglion was from 1:1,000,000 to 1:10,000. Throughout the experiments the preganglionic nerves were stimulated at a low frequency and the action potentials in the postganglionic fibers were recorded. We never observed any effect with the low concentrations of eserine and only a block of synaptic transmission with high concentrations.

It is to be emphasized, however, that our experiments need not be considered as opposed to those of Feldberg and Vartianen. They perfused the ganglion with Locke's fluid containing eserine while in our work the ganglion was circulated with blood. Our negative results may be due to the fact that with blood circulating through the ganglion so much eserine is needed that the ganglion is inactivated before the concentration of eserine is sufficient to protect the acetylcholine.

Bathing the ganglion with acetylcholine causes a marked discharge of postganglionic impulses.

### 8219 P

## Response of a Sympathetic Ganglion to High Frequency Stimulation.*

D. W. BRONK and R. J. PUMPHREY.

*From the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania.*

If a preganglionic mammalian nerve is stimulated at a low frequency, each volley of impulses initiates a fairly well synchronized discharge in the postganglionic nerve.[1] When the rate of stimulation is increased to 30 or 40 per second there is a progressive de-

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* Aided by a grant from the Committee on Grants-in-Aid of the National Research Council.

[1] Bronk, D. W., Tower, S. S., and Solandt, D. Y., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1659.

crease in the height of the postganglionic spike potential. This apparent failure in the capacity of the ganglion to transmit impulses at a high frequency is a property of the synapse which must be taken into consideration in the formulation of any theory which attempts to explain the mechanism of synaptic transmission.

All of our experiments were performed on cats in a room maintained at close to body temperature. The pregaglionic fibers in the third or fourth thoracic root were stimulated by means of a thyratron stimulator and the impulse discharge from the stellate ganglion was recorded in the inferior cardiac nerve.

The effect which we are considering can be demonstrated by stimulating the preganglionic nerve at a frequency of about 60 per second. The successive postganglionic spike potentials rapidly decrease in height and after a variable number of stimuli they can no longer be detected at ordinary amplifications. There is no such rapid failure at these frequencies of the spike potential in either the pre- or postganglionic nerves when they are stimulated directly. This suggests that the ganglion blocks the transmission of impulses at high frequencies.

Two observations argue against such a conclusion. In the first place we have found that stimulation of the preganglionic fibers at frequencies as high as 120 per second produces cardiac acceleration throughout long periods of stimulation. In the second place, the electrical record of postganglionic activity during such stimulation frequently shows a negative displacement of the base line which continues until the end of the stimulus. It would appear therefore that there is continued activity in the postganglionic fibers despite the absence of the usual spike potential.

These several observations can be explained by assuming that the impulses become so scattered or temporally dispersed in the many postganglionic fibers that they no longer reveal themselves as synchronized spike potentials. We have shown that this is indeed the case by cutting down the postganglionic trunk to a few fibers and recording the activity in those fibres during high frequency preganglionic stimulation. We thus found that the initial preganglionic volleys set up well synchronized volleys of postganglionic impulses but that the synchronization becomes rapidly less until there is a completely random discharge in the various fibers. At certain frequencies of stimulation there is at first a synchronized response of the postganglionic fibers to every second or third or fourth preganglionic volley following which the discharge becomes more and more asynchronous.

This altered property of the ganglion develops more rapidly at high frequencies of stimulation than at low and persists for many seconds following the end of the stimulus. After various intervals of rest following a period of high frequency stimulation we have tested the transmission through the ganglion by repeating the high frequency stimulation. The initial postganglionic spike is again maximal after a recovery period of less than a second but a minute or more may be required before the tenth or twentieth impulse is again as large as it was in the initial series. At low temperatures the progressive decrease in height of the synchronized spike potential is more rapid and the recovery of the capacity for synchronized activity is much delayed.

We suggest that these results may be due to an increase in the refractory period of the ganglion cells which is different for the various cells. Or one may assume *2* types of synaptic transmission, the one which is responsible for the grouped discharge failing more rapidly than that which produces the asynchronous firing. But these are speculations which must be tested by additional experiments.

### 8220 P

## Improvement in Female Sex Hormone Blood Test in Cyclical Menstruating Women and in Pregnancy Blood.

ROBERT T. FRANK AND MORRIS A. GOLDBERGER.

*From the Gynecological Service and the Laboratories, Mount Sinai Hospital, New York City.*

Advance in the chemistry of the female sex hormone or estrogenic factor—theelin, oestrin, etc.—has shown that some of the estrogenic compounds are ether insoluble. In consequence, we have returned to a modification of our earliest technic in which alcohol extraction was used.[1] By desiccating the venous blood specimen as heretofore with anhydrous sodium sulphate and then extracting with 95% alcohol, instead of ether, a great increase in estrogenic activity of the extract was noted.

The technic permits the accurate assay of a single specimen, the amount of extract employed in each animal depending upon the time of the cycle. By the old method[2] 40 cc. of blood give a negative reaction until the week preceding the onset of the menses is reached. By the new method, 40 cc. of blood even immediately after menstruation gives a positive reading.

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[1] Frank, R. T., and Goldberger, M. A., *J. A. M. A.*, 1926, **87**, 1719.

[2] Frank, R. T., and Goldberger, M. A., *J. A. M. A.*, 1928, **90**, 376.

1. The new technic requires 50 cc. of blood, dehydrated in anhydrous sodium sulphate. 2. The powder is twice extracted with 200 cc. of 95% alcohol. 3. The alcohol fractions are combined and evaporated to dryness on a water bath. 4. The residuum is taken up in 5 cc. of olive oil and injected into spayed mice. 5. The bio-assay is according to the Allen and Doisy method.

*Cyclical.* With the refinement of method just described, the bloods of 13 patients with normal menstrual cycles, have been examined.

By the old method, 40 cc. of blood were found to contain a mouse unit of estrogenic substance from the 7th day preceding menstruation, to the onset of the period, in the great majority of patients. Before this time the tests usually proved negative.

With the new method, it was found that 40 cc. of blood regularly give a positive reaction some 21 days before the onset of the menses; that 30 cc. are positive between the 21st and 14th day; that 20 to 10 cc. are positive from the 7th day preceding the period until menstruation.

The preliminary curves drawn on the basis of these findings correspond in every way with those of our previous reports, the difference being in the increased delicacy of the reaction.

*Pregnancy.* In our previous titrations of pregnancy blood[3, 4] we were unable to obtain a positive estrogenic reaction in 40 cc. of vein blood before the 8th week of pregnancy. By means of the new method, a positive estrogenic reaction is obtainable as early as the 3rd week of pregnancy with from 10 to 15 cc. of blood. In one patient 9 days overdue, the Friedman test already being positive, a plus 3 was obtained with 8 cc. of blood. By the 6th week of pregnancy, the reaction with 8 to 10 cc. of blood is regularly positive.

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[3] Frank, R. T., and Goldberger, M. A., *J. A. M. A.*, 1926, **87**, 1719.

[4] Frank, R. T., and Goldberger, M. A., *J. A. M. A.*, 1926, **90**, 106.

### 8221 P

## Significance of Estrogenic Factor in Tumors and Tissues of the Human Female.

ROBERT T. FRANK, MORRIS A. GOLDBERGER, UDALL J. SALMON* AND REUBEN FRIEDMAN.

*From the Laboratories, Mount Sinai Hospital, New York City.*

Considerable attention has been focussed on the presence and possible significance of the estrogenic factor in certain ovarian and other tumors,[1] in adenomata of the breast,[2] and in fibroids of the uterus.[3]

In previous publications we have emphasized the presence of the estrogenic factor in the follicle fluid, in certain cysts of the ovary, in malignant tumors of children in which premature puberty had occurred.

In none of these reports have control tissues been examined except the liver. We have now assayed the psoas muscle of 4 normal cyclical females and have there found the presence of large amounts of estrogenic factor varying in the different cases from 800 to 8000 M.U. kg. when calculated to a dry weight as Lewis and Geschickter have done. In one case, where the material was obtained at autopsy shortly after death from embolism, the psoas muscle titered 2000 M.U. kg., the *normal* uterus, 8000 M.U. kg. (intermenstrual).

Therefore it appears unwarranted to base general conclusions on the mere presence of the estrogenic factor in abnormal tissues unless further study of other normal tissues shows that the estrogenic factor is not widely distributed throughout the body of the human female. Our observation, if found to apply only to the muscles, may also indicate that the estrogenic factor accumulates in these depots in a fashion similar to the deposition of certain other products (glycogen) to be liberated and utilized when required by the organism.

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* Joseph Brettauer Research Fellow.

[1] Frank, R. T., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 1204.

[2] Lewis, D., and Geschickter, C. F., *J. A. M. A.*, 1934, **103**, 1212.

[3] Lewis, D., and Geschickter, C. F., *J. A. M. A*, 1935, **104**, 45.

## 8222 P

# Determination of Luteinizing and Follicle-Stimulating Principles in Castrate and Menopause Urine.

ROBERT. T. FRANK, U. J. SALMON,* AND REUBEN FRIEDMAN.

*From the Gynecological Service and Laboratories, Mount Sinai Hospital, New York City.*

*Method.* By the following method it has been possible to demonstrate the presence of luteinizing (in 62.5% of 16 women) as well as follicle-stimulating principle in the urine of both castrates and spontaneous menopause.

Two hundred cc. of urine is taken from a fresh 24 hours' specimen and acidified with concentrated acetic acid to pH 3.5 (Congo Red). Four volumes of cold acetone are added to the urine, shaken vigorously and allowed to stand overnight in the refrigerator. The supernatant fluid is poured off and the precipitate extracted with weak NaOH. The pH of the mixture is adjusted to between 8 and 8.5, the residual precipitate stirred thoroughly, the mixture centrifuged, and the final precipitate discarded as the supernatant fluid contains the gonadotropic principle. The fluid is then adjusted to pH 7 with dilute acetic acid. The equivalent of 100, 50, and 25 cc. is injected into immature rats weighing 22 to 24 gm. The injections in each rat are divided into 5 doses, given over 3 days. The animals are autopsied at the end of 96 hours. The ovaries are examined in serial section.

The same procedure can be performed using alcohol instead of acetone. Acetone is preferable to alcohol as no deterioration or destruction of the hormone occurs on standing, whereas deterioration of the gonadotropic principle occurs when alcohol is used.

The opinion is gaining ground that "the principle in castrate or menopause urine seems to be identical with the follicle-stimulating fraction prepared from the pituitary glands."[1] However, it should be emphasized that Lassen and Brandstrup[2] reported a luteinizing reaction in the urine (diagnosis by gross inspection of the mouse ovaries), and Fluhman[3] in the blood of such patients.

Katzman and Doisy's benzoic acid precipitation method[4] does not extract the factor found in the menopause or castrate urine. By using our new method, 16 cases, partly surgical castrates, X-ray castrations, and spontaneous menopause were studied.

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* Joseph Brettauer Research Fellow.

[1] Smith, P. E., *J. A. M. A.*, 1935, **104**, 553.

[2] Lassen, H. C. A., and Brandstrup, E., *Acta Scand. Obst. et Gynec.*, 1934, **14**, 89.

[3] Fluhman, C. F., *J. A. M. A.*, 1929, **93**, 672.

[4] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **106**, 125.

The luteinizing factor was found in the urine of 6 out of 9 surgical castrates, in 3 of 5 spontaneous menopause cases and in 1 of 2 X-ray castrates, 62.5% of 16 cases. Luteinization was produced with from 25 to 100 cc. of urine. With diminishing quantities of extract, the luteinizing reaction disappeared, the follicle-stimulating effect becoming more marked. Fluctuation in excretion was noted in individual women, equal quantities, varying between strong luteinizing, follicle-stimulating and negative effect.

In 3 cases examined, the luteinizing factor was demonstrated in the blood.

No demonstrable correlation between the symptoms complained of and the quantity or quality of the gonadotropic hormone excreted in the menopause, whether spontaneous or induced (surgical or X-ray) could be noted. Two patients who had no symptoms, excreted 10 to 40 R.U. per liter of both factors. Four cases with mild symptoms excreted 10 to 55 R.U. per liter of only follicle-stimulating factor. In the 10 cases with severe symptoms, no correlation was apparent.

## 8223 P

## Micromethod for Determining Insect Hemolymph Specific Gravity (Periplaneta americana Linn).*

J. FRANKLIN YEAGER and R. W. FAY.

*From the Department of Zoology and Entomology, Iowa State College.*

This is a preliminary report of a falling drop method (based on Stokes's law) that permits the determination of the specific gravity (S.G.) of a single drop of insect hemolymph (4.85 mm.$^3$ or less) within the temperature range of 15°C.-40°C., with an average accuracy of ±0.0021 (1 determination) or ±0.0008 (mean of 10 determinations), and with an average determination time of about 8 minutes. The diameter of the hemolymph drop at a beeswax-mineral oil surface is measured with a calibrated ocular micrometer scale. The hemolymph drop, surrounded by oil, is sucked into a

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* This work was done under a grant from the Rockefeller Fluid Research Fund administered by Iowa State College.

glass tube and thus transferred to the sedimentation cylinder. The time required for the drop to sediment through a given falling distance in mineral oil is measured and converted to falling time at 25°C. by means of conversion factors calculated from the equation

$$t_2 = \left( \frac{n_2 k_2 (p_s - p_m)_1}{n_1 k_1 (p_s - p_m)_2} \right) t_1 \qquad (1)$$

in which n is the viscosity of the oil medium, $p_s$ is the S.G. of the drop, $p_m$ is the S.G. of the oil medium, t is the falling time and the subscripts $_1$ and $_2$ refer respectively to the experimental temperature and to 25°C. The correction factor, k, for fluidity of the drop, was defined by Hadamard[1] as $k = (2n + 3n')/(3n + 3n')$, where n is the viscosity of the medium and $n'$ is that of the drop. This variable factor was found to be $k_2 = 0.6692$ at 25°C. By means of curves that show falling time-specific gravity difference relationships for drops of various sizes at 25°C., the drop-medium S.G. difference is found and is added to the S.G. of the medium to obtain that of the hemolymph at 25°C. The curves were calculated by means of the equation

$$t_2 = \frac{9ns(1 + 2.4\ d/D)\ (1 + 1.65\ d/h)k}{2g\ a^2\ (p_s - p_m)} \qquad (2)$$

which is derived from Stokes's law

$$V = \frac{s}{t} = \frac{2g\ a^2\ (p_s - p_m)}{9n} \qquad (3)$$

In these equations, d is drop diameter, D is diameter of the container of the medium, h is the height of the column of the medium, g is the gravitational constant, a is the radius of the drop, s is the falling distance, V is the falling velocity and n, k, $p_s$, $p_m$ and $t_2$ have the meanings already given them. The factors $(1 + 2.4\ d/D)$ and $(1 + 1.65\ d/h)$ correct for side wall and end effects of the container of the medium.[2]

The S.G. values of single hemolymph drops from 250 cockroaches, *P. americana,* were determined with this method. The means and standard deviations are 1.0163 ± 0.0112 (entire group), 1.0150 ± 0.0113 (150 adults), 1.0182 ± 0.0108 (100 nymphs), 1.0149 ± 0.0114 (125 males) and 1.0175 ± 0.0109 (125 females). The adult-nymph difference was and the male-female difference was not found to be statistically significant.

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[1] Hadamard, J. S., *C. R. Acad. Sci.*, 1911, **152**, 1735.

[2] Barr, G., A Monograph of Viscometry, 1931.

This method is applicable to hemolymphs of other insects and other invertebrates and to animal or plant fluids available only in minute quantities. Successive determinations on individual insects under varying conditions can be made with no serious injury to the animal.

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### 8224 C

## Fasting Blood Sugar in Rats.

ESTHER M. GREISHEIMER, ELMER GEORGE, AND LLOYD GILMAN.

*From the Department of Physiology, University of Minnesota.*

It was found that the blood sugar during fasting falls more rapidly in women than in men.[1]

The study has been continued on rats. The animals were divided into 3 groups; the first group was fasted 12 hours, the second, 24 hours, and the third, 42 hours. Water was allowed during the fast. At the close of the fasting period, the rats were decapitated, and the blood sugar determined in duplicate by the Shaffer-Somogyi method.

The values found are shown in Table I.

TABLE I.

| Fasting period | | Males | No. of rats | Females | No. of rats |
|---|---|---|---|---|---|
| 12 hours | Min. | 79.69 | | 77.36 | |
| | Max. | 100.19 | | 101.82 | |
| | Aver. | 89.96 | 9 | 94.81 | 12 |
| 24 hours | Min. | 70.60 | | 61.74 | |
| | Max. | 85.28 | | 89.94 | |
| | Aver. | 78.83 | 7 | 77.07 | 14 |
| 42 hours | Min. | 65.47 | | 52.66 | |
| | Max. | 75.72 | | 82.02 | |
| | Aver. | 71.14 | 3 | 68.45 | 17 |

The total fall for the female rats was 27.8%; of this 67% occurred between 12 and 24 hours; the total fall for the male rats was 20.9%; of this, 59% occurred between 12 and 24 hours.

These results agree with those found in men and women in that the blood sugar falls more rapidly in females during fasting.

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[1] Greisheimer, E. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1067.

# SECRETARY'S ANNUAL REPORT

## April 1, 1934 to April 1, 1935

### PROCEEDINGS

The finances of the Society have permitted a charge for excess space fifty per cent below cost.

The Council authorized copyrighting of all issues of the PROCEEDINGS.

There were 623 manuscripts published during the fiscal year, of which 49 per cent were preliminary and 51 per cent complete.

### MEMBERSHIP

| | | |
|---|---|---|
| Members, March 31, 1934 | 1207 | |
| Elected during year | 72 | |
| Honorary Members | 9 | |
| Total | | 1288 |
| Resignations | 7 | |
| Deaths | 9 | |
| Arrears | 6 | 22 |
| Total Membership, March 31, 1935 | | 1266 |

| | 1926 | 1927 | 1928 | 1929 | 1930 | 1931 | 1932 | 1933 | 1934 | 1935 |
|---|---|---|---|---|---|---|---|---|---|---|
| Membership | 779 | 851 | 936 | 986 | 1026 | 1062 | 1138 | 1146 | 1218 | 1266 |

### SUBSCRIPTIONS

| | |
|---|---|
| Subscriptions, March 31, 1934 | 432 |
| Subscriptions, March 31, 1935 | 449 |
| Total No. of Proceedings distributed 3/31/34 | 1650 |
| Total No. of Proceedings distributed 3/31/35 | 1715 |

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| 1905-06 | E. B. Wilson | E. K. Dunham | W. J. Gies | G. N. Calkins |
| 1906-07 | S. Flexner | E. K. Dunham | W. J. Gies | G. N. Calkins |
| 1907-08 | S. Flexner | T. H. Morgan | W. J. Gies | G. N. Calkins |
| 1908-09 | F. S. Lee | T. H. Morgan | W. J. Gies | G. Lusk |
| 1909-10 | F. S. Lee | W. J. Gies | E. L. Opie | G. Lusk |
| 1910-11 | T. H. Morgan | W. J. Gies | E. L. Opie | G. Lusk |
| 1911-12 | T. H. Morgan | P. A. Levene | G. B. Wallace | G. Lusk |
| 1912-13 | J. Ewing | P. A. Levene | G. B. Wallace | C. Norris |
| 1913-14 | J. Ewing | C. W. Field | H. C. Jackson | C. Norris |
| 1914-15 | G. Lusk | W. J. Gies | H. C. Jackson | J. R. Murlin |
| 1915-16 | G. Lusk | G. N. Calkins | H. C. Jackson | |
| 1916-17 | J. Loeb | W. J. Gies | H. C. Jackson | |
| 1917-18 | W. J. Gies | J. Auer | H. C. Jackson | |
| 1918-19 | W. J. Gies | J. Auer | H. C. Jackson | |
| 1919-20 | G. N. Calkins | G. B. Wallace | H. C. Jackson | |
| 1920-21 | G. N. Calkins | G. B. Wallace | H. C. Jackson | |
| 1921-22 | G. B. Wallace | J. W. Jobling | H. C. Jackson | |
| 1922-23 | G. B. Wallace | J. W. Jobling | H. C. Jackson | |
| 1923-24 | H. C. Jackson | J. W. Jobling | V. C. Myers | |
| 1924-25 | H. C. Jackson | J. W. Jobling | A. J. Goldforb | |
| 1925-26 | J. W. Jobling | S. R. Benedict | A. J. Goldforb | |
| 1926-27 | J. W. Jobling | S. R. Benedict | A. J. Goldforb | |
| 1927-28 | S. R. Benedict | P. Rous | A. J. Goldforb | |
| 1928-29 | S. R. Benedict | P. Rous | A. J. Goldforb | |
| 1929-30 | P. Rous | D. Marine | A. J. Goldforb | |
| 1930-31 | P. Rous | D. J. Edwards | A. J. Goldforb | |
| 1931-32 | D. J. Edwards | A. R. Dochez | A. J. Goldforb | |
| 1932-33 | A. R. Dochez | E. L. Opie | A. J. Goldforb | |
| 1933-34 | A. R. Dochez | E. L. Opie | A. J. Goldforb | |
| 1934-35 | E. L. Opie | P. E. Smith | A. J. Goldforb | |

## SECTIONS

### *Cleveland, Ohio*

Chairman: J. A. Doull. Secretary: A. R. Moritz. Members: 39.

Meetings: Institute of Pathology, Western Reserve University, October 12, 1934.

Institute of Pathology, Western Reserve University, November 9, 1934.
Institute of Pathology, Western Reserve University, December 14, 1934.
Institute of Pathology, Western Reserve University, January 11, 1935.
Institute of Pathology, Western Reserve University, February 8, 1935.
Institute of Pathology, Western Reserve University, March 8, 1935.
Institute of Pathology, Western Reserve University, May 10, 1935.

### *District of Columbia**

Chairman: E. B. Meigs. Secretary: C. J. Stucky. Members: 24.
Meetings: George Washington University, December 21, 1934.
Cosmos Club, Washington, February 25, 1935.
George Washington University, May 10, 1935.

### *Illinois*

Chairman: W. Thalhimer. Secretary: W. L. Palmer. Members: 114.
Meetings: University of Chicago, October 23, 1934.
University of Illinois College of Medicine, January 8, 1935.
Northwestern University Medical School, April 28, 1935.
Loyola University Medical School, May 21, 1935.

### *Iowa*

Chairman: H. M. Hines. Secretary: J. A. Greene. Members: 35.
Meetings: State University of Iowa, February 19, 1935.
State University of Iowa, May 17, 1935.

### *Minnesota*

Chairman: F. H. Scott. Secretary: F. H. Scott. Members: 46.
Meetings: University of Minnesota, March 20, 1935.
University of Minnesota, April 17, 1935.
University of Minnesota, May 23, 1935.

### *Missouri*

Chairman: E. A. Doisy. Secretary: G. H. Bishop. Members: 45.
Meetings: St. Louis University Medical School, November 14, 1934.
Washington University Medical School, January 9, 1935.
St. Louis University Medical School, February 13, 1935.
Washington University Medical School, May 15, 1935.

### *New York*

Chairman: E. L. Opie. Secretary: A. J. Goldforb. Members: 704.
Meetings: New York Academy of Medicine, October 17, 1934.
New York Academy of Medicine, November 21, 1934.
New York Academy of Medicine, December 19, 1934.
New York Academy of Medicine, January 16, 1935.

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*This new Section was authorized October, 1934.

New York Academy of Medicine, February 20, 1935.
New York Academy of Medicine, March 20, 1935.
New York Academy of Medicine, April 17, 1935.
New York Academy of Medicine, May 15, 1935.

### *Pacific Coast*

Chairman: W. H. Manwaring. Secretary: D. M. Greenberg. Members: 80.
Meetings: Stanford University, October 20, 1934.
University of California Hospital, December 12, 1934.
Stanford University Hospital, February 6, 1935.
Mount Zion Hospital, San Francisco, March 20, 1935.
University of California, May 4, 1935.

### *Peiping (China)*

Chairman: C. N. Frazier. Secretary: H. C. Chang. Members: 24.
Meetings: Peiping Union Medical College, October 24, 1934.
Peiping Union Medical College, January 30, 1935.
Peiping Union Medical College, April 3, 1935.

### *Southern*

Chairman: H. Cummins. Secretary: A. Ochsner. Members: 32.
Meetings: San Antonio, Texas, November 14, 1934.
Tulane University, February 15, 1935.
Tulane University, May 17, 1935.

### *Southern California*

Chairman: T. D. Beckwith. Secretary: D. R. Drury. Members: 27.
Meetings: Los Angeles County General Hospital, November 27, 1934.
La Jolla, California, December 28, 1934.
Los Angeles County Medical Association, April 11, 1935.

### *Western New York*

Chairman: J. A. Dye. Secretary: G. H. Maughan. Members: 52.
Meetings: Cornell University, October 20, 1934.
University of Rochester, December 15, 1934.
University of Buffalo, February 23, 1935.
Syracuse University, May 4, 1935.

### *Wisconsin*

Chairman: E. L. Sevringhaus. Secretary: M. R. Irwin. Members: 35.
Meetings: Marquette University, Milwaukee, May 22, 1935.

## DEATHS OF MEMBERS

The Council records with regret the deaths of the following members: Doctors C. W. Baldridge, W. D. Halliburton, E. G. Martin, A. W. Rowe, Theobald Smith, H. C. Stevens, R. W. Thatcher.

A. J. GOLDFORB,
*Secretary.*

# TREASURER'S REPORT

## April 1, 1934 to April 1, 1935

### RECEIPTS

| | | |
|---|---|---|
| Balance on Hand, April 1, 1934 | | $ 5,087.20 |
| Income, 1934-35: | | |
| Dues | $ 5,044.15 | |
| Reprints | 2,921.46 | |
| Excess Space | 1,452.68 | |
| Cuts | 545.91 | |
| Subscriptions | 2,678.24 | |
| Back Numbers Sold | 508.96 | |
| Miscellaneous | 48.23 | |
| Total Annual Income | | $13,199.63 |
| Total Cash Available, April 1, 1934-April 1, 1935 | | $18,286.83 |
| Publication Cost of Proceedings: | | |
| Printing | $ 5,802.60* | |
| Reprints | 2,785.26† | |
| Cuts | 571.46‡ | |
| | | $ 9,159.32 |
| Administrative Expenses: | | |
| Office Supplies, Equipment, Postage, Telephone | $ 912.46 | |
| Salaries | 1,938.00 | |
| Storage and Insurance | 89.25 | |
| Refunds | 20.00 | |
| Miscellaneous | 47.46 | |
| | | $ 3,007.17 |
| Total Annual Disbursements | | $12,166.49 |
| Annual Surplus Transferred to Surplus Fund | | 1,106.56 |
| Cash Balance, April 1, 1935 | | 5,013.78 |
| Total | | $18,286.83 |

### SUMMARY

| | | |
|---|---|---|
| *Income* (net) | | $13,199.63 |
| *Disbursements* (net) | $12,166.49 | |
| Unpaid bills | 925.24 | |
| | | $13,091.73 |
| *Surplus for year* | | $ 107.90 |

*Plus $613.31 not yet paid.

†Plus $256.53 not yet paid.

‡Plus $ 55.40 not yet paid.

## FUNDS

### *Endowment Fund*

| | | |
|---|---|---|
| April 1, 1934 | $14,440.51 | |
| Interest to April 1, 1935 | 414.99 | |
| Total | | $14,855.50 |

| | | |
|---|---|---|
| Invested in New York Title and Mortgage Co. | $ 6,000.00 | |
| Invested in Title Guarantee and Trust Co. | 2,500.00 | |
| Invested in Lawyers Mortgage Co. | 1,500.00 | |
| Invested in Railroad Cooperative Bldg. and Loan | 1,007.24 | |
| Invested in Bowery Savings Bank | 818.36 | |
| Invested in Industrial Bonds (Purchase price) | 3,029.90 | |
| (Present value $3,215.00) | | $14,855.50 |

### *Special Surplus Fund*

| | | |
|---|---|---|
| April 1, 1934 | $ 7,883.21 | |
| 1933-34 Surplus | 1,106.56 | |
| Interest to April 1, 1935 | 335.09 | |
| Total | | $ 9,324.86 |

| | | |
|---|---|---|
| Invested in Title Guarantee and Trust Co. | $ 2,900.00 | |
| Invested in Railroad Cooperative Bldg. and Loan | 2,680.13 | |
| Invested in Bowery Savings Bank | 571.26 | |
| Invested in Industrial Bonds (Purchase price) | 3,173.47 | |
| (Present value $3,386.25) | | $ 9,324.86 |

### *Life Membership Fund*

| | |
|---|---|
| Invested in Railroad Cooperative Bldg. and Loan | $ 75.00 |

### MEMO

| | |
|---|---|
| Bills payable | $ 876.91 |
| Bills receivable | $ 823.89 |

## **Auditors' Report**

April 16, 1935

We have examined the Treasurer's report and have verified the statements therein against the books of the Society. We are convinced that the records of financial transactions are in good order and accurately kept.

(Signed) W. J. MACNEAL
PHILIP E. SMITH
FLORENCE R. SABIN.

# MEMBERS' LIST (Alphabetical)

## HONORARY MEMBERS

| | |
|---|---|
| Councilman, William T. | Harvard University |
| Howell, William H. | Johns Hopkins University |
| Lombard, Warren P. | University of Michigan |
| Porter, William | Harvard University |
| Reichert, E. T. | University of Pennsylvania |
| Richet, Charles | Paris, France |
| Schaffer, E. S. | Edinburgh, Scotland |
| Von Muller, Friedrich | Munich, Germany |
| Wilson, Edmund B. | Columbia University |

## MEMBERS

| | |
|---|---|
| Abbott, Alexander C. | University of Pennsylvania |
| Abel, John J. | Johns Hopkins University |
| Adams, A. Elizabeth | Mount Holyoke College |
| Adams, William E. | University of Chicago |
| Addis, Thomas | Medical School, Stanford University |
| Adler, Herman M. | Berkeley, California |
| Adolph, E. F. | University of Rochester Medical School |
| Adolph, W. H. | Peiping, China |
| Alexander, Harry L. | Washington University |
| Allen, Bennet M. | University of California |
| Allen, Edgar | Yale University |
| Allen, Ezra | N. Y. Homeopathic Medical College |
| Allen, William F. | University of Oregon |
| Alles, G. A. | Los Angeles, California |
| Alsberg, Carl L. | Stanford University |
| Alt, Howard L. | Northwestern University Medical School |
| Althausen, T. L. | University of California Medical School |
| Alvarez, Walter C. | Mayo Clinic |
| Amberg, Samuel | Mayo Clinic |
| Amberson, W. R. | University of Tennessee Medical School |
| Amoss, Harold L. | Rockefeller Institute |
| Andersen, Dorothy H. | College of Physicians and Surgeons |
| Anderson, H. H. | University of California Medical School |
| Anderson, John E. | University of Minnesota |
| Anderson, John F. | E. R. Squibb & Son |
| Anderson, Rudolph J. | Yale University |
| Anderson, William E. | Yale University |
| Andrews, Edmund | University of Chicago |
| Anson, Mortimer L. | Rockefeller Institute, Princeton, N. J. |
| Arnold, Lloyd | University of Illinois |

| | |
|---|---|
| Aronson, J. D. | Henry Phipps Institute |
| Asdell, S. A. | Cornell University |
| Asher, Leon | Berne, Switzerland |
| Ashman, Richard | Louisiana State Univ. Medical Center |
| Atchley, D. W. | Presbyterian Hospital, New York City |
| Atwell, Wayne J. | University of Buffalo, N. Y. |
| Aub, Joseph C. | Huntington Memorial Hospital, Boston |
| Auer, John | St. Louis University |
| Austin, J. Harold | University of Pennsylvania |
| Austin, W. C. | Loyola University |
| Avery, O. T. | Rockefeller Institute, N. Y. City |
| | |
| Babkin, Boris P. | McGill University |
| Bachem, Albert | University of Illinois Medical College |
| Baehr, George | Mt. Sinai Hospital, N. Y. City |
| Bagg, Halsey J. | Memorial Hospital, N. Y. City |
| Bailey, Cameron V. | N. Y. Post-Graduate Medical School |
| Baitsell, George A. | Yale University |
| Bakwin, Harry | N. Y. University and Bellevue Medical College |
| Baldwin, W. Manning | Albany Medical College |
| Ball, G. H. | University of California, Los Angeles |
| Balls, A. K. | U. S. Dept. of Agriculture |
| Banta, A. M. | Brown University |
| Banting, Frederick G. | University of Toronto |
| Barach, Alvin L. | College of Physicians and Surgeons |
| Barber, W. Howard | New York University Med. School |
| Barbour, Henry G. | Yale University |
| Bardeen, Charles R. | University of Wisconsin |
| Barer, Adelaide P. | State University of Iowa |
| Barlow, Orphus W. | Western Reserve University |
| Barnett, George D. | Stanford University |
| Barr, David P. | Washington University |
| Barron, E. S. G. | University of Chicago |
| Barth, L. G. | Columbia University |
| Bass, Charles | Tulane University |
| Bast, T. H. | University of Wisconsin |
| Bates, R. W. | Carnegie Institution of Washington |
| Bauer, J. H. | Rockefeller Institute |
| Bauman, Louis | Presbyterian Hospital, N. Y. City |
| Baumann, E. J. | Montefiore Hospital, N. Y. City |
| Baumberger, J. Percy | Stanford University |
| Bayne-Jones, S. | Yale University |
| Bazett, H. C. | University of Pennsylvania |
| Bean, John W. | University of Michigan |
| Beard, H. H. | Louisiana State Univ. Medical Center |
| Beck, Claude S. | Western Reserve University |
| Becker, E. R. | Iowa State College |
| Beckman, Harry | Marquette University Medical School |
| Beckwith, T. D. | University of California, Los Angeles |
| Behre, Jeannette A. | Cornell University Medical College |

| | |
|---|---|
| Belding, David L. | Boston University |
| Bellamy, Arthur W. | University of California, Los Angeles |
| Benedict, S. R. | Cornell University Medical College, N. Y. City |
| Benton, Anne G. | Vassar College |
| Berg, B. N. | Columbia University |
| Berg, C. P. | State University of Iowa |
| Berg, William N. | New York City |
| Bergeim, Olaf | University of Illinois |
| Bergey, David H. | National Drug Co., Philadelphia |
| Bergmann, Max | Rockefeller Institute |
| Bernhard, Adolph | Lenox Hill Hospital, N. Y. City |
| Bernthal, T. G. | University of Michigan |
| Berry, George P. | University of Rochester Medical School |
| Beutner, R. | University of Louisville |
| Bieter, Raymond N. | University of Minnesota |
| Bills, C. E. | Mead, Johnson and Co., Evansville, Ind. |
| Bing, Franklin C. | Western Reserve University |
| Binger, Carl A. L. | Rockefeller Institute, N. Y. City |
| Birkhaug, Konrad E. | Pasteur Institute, Paris, France |
| Bishop, George H. | Webster Groves, Mo. |
| Bishop, Katharine S. | University of California |
| Blackfan, K. D. | Harvard Medical School |
| Blair, John E. | Hospital for Joint Diseases, N. Y. City |
| Blake, F. G. | Yale University |
| Blakeslee, Albert F. | Station for Exp. Evolution, Cold Spring Harbor, N. Y. |
| Blalock, Alfred | Vanderbilt University Medical School |
| Blatherwick, Norman R. | Metropolitan Life Insurance Co., New York City |
| Blau, Nathan F. | Cornell University Medical College |
| Blinks, L. R. | Stanford University |
| Bliss, Sidney | Tulane University |
| Bloch, Robert G. | University of Chicago |
| Block, Richard J. | N. Y. State Psychiatric Institute |
| Bloom, William | University of Chicago |
| Bloomfield, A. L. | Stanford University Medical School |
| Bloor, W. R. | University of Rochester |
| Blum, Harold F. | University of California |
| Blumgart, H. L. | Beth Israel Hospital, Boston |
| Bodansky, A. | New York City |
| Bodansky, Meyer | John Sealy Hospital, Galveston, Texas |
| Bodine, J. H. | State University of Iowa |
| Bodo, Richard | N. Y. University and Bellevue Med. College |
| Bollman, Jesse L. | Mayo Clinic |
| Boor, Alden K. | University of Chicago |
| Boothby, Walter M. | Kahler Hospital, Rochester, Minn. |
| Boots, Ralph H. | Presbyterian Hospital, New York City |
| Borsook, Henry | California Institute of Technology |
| Bowen, B. D. | Buffalo General Hospital |
| Boyd, Theo. E. | Loyola University |
| Boyden, E. A. | University of Minnesota Medical School |

| | |
|---|---|
| Bradford, William L. | University of Rochester |
| Bradley, H. C. | University of Wisconsin |
| Brand, Erwin | New York State Psychiatric Institute |
| Branham, Sara E. | National Inst. of Health, Washington |
| Brewer, Robert K. | Syracuse University |
| Briggs, A. P. | St. Louis University |
| Brodie, Maurice | Dept. of Health, New York City |
| Bronfenbrenner, J. | Washington University |
| Bronk, D. W. | University of Pennsylvania |
| Brooks, Clyde | Louisiana State University Medical Center |
| Brooks, Harlow | New York University |
| Brooks, Matilda M. | University of California |
| Brooks, S. C. | University of California |
| Broun, G. O. | St. Louis University Medical School |
| Brown, E. D. | University of Minnesota |
| Brown, J. Howard | Johns Hopkins University |
| Brown, John B. | Ohio State University |
| Brown, L. A. | Transylvania College, Lexington, Ky. |
| Brown, Rachel | N. Y. State Dept. of Health |
| Brown, Wade H. | Rockefeller Institute, N. Y. City |
| Bruger, Maurice | N. Y. Post-Graduate Medical School |
| Buchanan, Robert E. | Iowa State College |
| Buchbinder, W. C. | Michael Reese Hospital, Chicago |
| Buell, Mary V. | Johns Hopkins University |
| Bulger, H. A. | Washington University |
| Bullowa, J. G. M. | Harlem Hospital, N. Y. City |
| Bunting, C. H. | University of Wisconsin |
| Bunting, R. W. | University of Michigan |
| Burch, John G. | Vanderbilt University Medical School |
| Burget, G. E. | University of Oregon Medical Scrool |
| Burky, Earl L. | Johns Hopkins Hospital |
| Burns, Robert K., Jr. | University of Rochester |
| Burr, George O. | University of Minnesota |
| Burr, Harold S. | Yale University |
| Burrows, M. T. | Pasadena, Calif. |
| Byerly, T. C. | U. S. Animal Exp. Farm, Beltsville, Md. |
| Byrne, Joseph | Fordham University |

| | |
|---|---|
| Calkins, Gary N. | Columbia University |
| Cannan, Robert K. | N. Y. University and Bellevue Med. College |
| Cannon, Paul R. | University of Chicago |
| Cannon, Walter B. | Harvard Medical School |
| Carey, E. J. | Marquette University Medical School |
| Carlson, A. J. | University of Chicago |
| Carmichael, E. B. | University of Alabama School of Medicine |
| Carruthers, Albert | Peiping Union Medical College |
| Carter, Edward P. | Johns Hopkins Hospital |
| Casey, Albert E. | University of Virginia |
| Cash, James R. | University of Virginia |

| | |
|---|---|
| Cattell, McKeen | Cornell University Medical College |
| Caulfeild, A. H. W. | University of Toronto |
| Cecil, R. L. | Cornell University Medical College |
| Chace, Arthur F. | N. Y. Post-Graduate Medical College |
| Chaikoff, I. L. | University of California |
| Chambers, Robert | New York University |
| Chambers, William H. | Cornell University Medical College |
| Chang, Hsi Chun | Peiping Union Medical College |
| Chang, Hsiao-Chien | Peiping Union Medical College |
| Charipper, H. A. | New York University |
| Cheer, S. N. | Central Hospital, Nanking, China |
| Chen, K. K. | Eli Lilly and Co., Indianapolis |
| Cheney, R. H. | Long Island University |
| Chidester, F. E. | University of Michigan |
| Child, C. M. | University of Chicago |
| Chittenden, R. H. | Yale University |
| Christian, Henry A. | Peter Bent Brigham Hospital |
| Christman, Adam A. | University of Michigan |
| Clark, A. J. | University of Edinburgh, Scotland |
| Clark, Guy W. | Lederle Lab., Pearl River, N. Y. |
| Clark, P. F. | University of Wisconsin |
| Clarke, Hans T. | College of Physicians and Surgeons |
| Claussen, S. W. | Strong Memorial Hospital, Rochester, N. Y. |
| Clawson, Benjamin J. | University of Minnesota |
| Clifton, Charles E. | Stanford University |
| Clowes, G. H. A. | Eli Lilly and Co., Indianapolis, Indiana |
| Coca, A. F. | Oradell, N. J. |
| Coghill, G. E. | Wistar Institute |
| Cohen, Barnett | Johns Hopkins Medical School |
| Cohen, Martin | N. Y. Post-Graduate Medical School |
| Cohen, Milton B. | St. Alexis Hospital, Cleveland |
| Cohn, A. E. | Rockefeller Institute, N. Y. City |
| Cohn, Isidore | New Orleans, La. |
| Cole, Arthur G. | University of Illinois Medical School |
| Cole, Harold H. | University of California, Davis |
| Cole, L. J. | University of Wisconsin |
| Cole, Rufus I. | Rockefeller Institute, N. Y. City |
| Cole, William H. | Rutgers University |
| Coleman, Warren | New York University Medical College |
| Collens, William S. | Brooklyn Jewish Hospital |
| Collett, Mary E. | Western Reserve University |
| Collier, William D. | St. Louis University |
| Collins, H. H. | Crafton, Pa. |
| Collip, J. B. | McGill University |
| Conklin, E. G. | Princeton University |
| Connor, Charles L. | University of California Medical School |
| Cook, Donald H. | School of Tropical Medicine, San Juan, Puerto Rico |
| Cook, S. F. | University of California |
| Cooke, J. V. | Washington University |
| Coombs, Helen C. | New York Homeopathic Medical College |

| | |
|---|---|
| Copenhaver, W. M. | Columbia University |
| Cori, Carl F. | Washington University |
| Corley, Ralph C. | Purdue University |
| Corner, George W. | University of Rochester |
| Corper, H. J. | National Jewish Hospital, Denver, Colo. |
| Cotui, F. W. | New York University and Bellevue Med. College |
| Coulter, Calvin B. | Columbia University |
| Cowdry, E. V. | Washington University |
| Cowgill, George R. | Yale University |
| Cowie, D. M. | University of Michigan |
| Craig, C. F. | Tulane University |
| Crampton, C. Ward | N. Y. Post-Graduate Medical School |
| Crandall, L. A., Jr. | Northwestern University |
| Crile, George W. | Western Reserve University |
| Crohn, Burrill B. | Mt. Sinai Hospital, N. Y. City |
| Cruickshank, E. W. H. | Dalhousie University, Halifax |
| Csonka, F. A. | U. S. Dept. of Agriculture, Washington, D. C. |
| Cullen, Glenn E. | Childrens Hospital, Cincinnati, O. |
| Cummins, Harold | Tulane University |
| Cunningham, R. S. | Vanderbilt University |
| Curtis, G. M. | Ohio State University |
| Curtis, Maynie R. | Columbia University |
| Cushing, Harvey W. | Yale University |
| Cutler, Elliott C. | Peter Bent Brigham Hospital, Boston |

| | |
|---|---|
| Dack, Gail M. | University of Chicago |
| Dakin, H. D. | Ossining, N. Y. |
| Danforth, Charles H. | Stanford University |
| Daniel, J. Frank | University of California |
| Daniels, Amy L. | University of Iowa |
| Danzer, Charles S. | N. Y. Homeopathic Medical College |
| Dautrebande, Lucien | University of Liege, Belgium |
| Davenport, C. B. | Sta. for Exp. Evolution, Cold Spring Harbor, N. Y. |
| Davenport, H. A. | Northwestern University Medical School |
| Davies, H. Whitridge | The University, Sydney, Australia |
| Davis, D. J. | Wilmette, Illinois |
| Dawson, James A. | College of the City of New York |
| Dawson, M. H. | Columbia University |
| Dawson, W. T. | University of Texas Medical School |
| Day, A. A. | Northwestern University Medical School |
| DeEds, Floyd | Stanford University Medical School |
| DeGraff, A. C. | N. Y. University Medical School |
| DeRenyi, G. S. | University of Pennsylvania |
| Derick, C. L. | Peter Bent Brigham Hospital, Boston |
| Detwiler, S. R. | Columbia University |
| Deuel, Harry J., Jr. | University of Southern California Medical School |
| Dick, George F. | University of Chicago |
| Dickson, E. C. | Stanford University Medical School |
| Dieckmann, W. J. | University of Chicago |

| | |
|---|---|
| Dienes, Louis | Massachusetts General Hospital, Boston |
| Dieuaide, Francis R. | Peiping Union Medical College, China |
| Doan, Charles A. | Ohio State University |
| Dochez, A. R. | Presbyterian Hospital, N. Y. City |
| Dock, William | Stanford University |
| Doisy, Edward A. | St. Louis University |
| Dolley, W. L., Jr. | University of Buffalo |
| Dominguez, R. | St. Luke's Hospital, Cleveland |
| Donaldson, H. H. | Wistar Institute, Philadelphia |
| Donaldson, J. C. | University of Pittsburgh |
| Dooley, M. S. | Syracuse University |
| Doull, J. A. | Western Reserve University |
| Downes, Helen R. | Memorial Hospital, New York City |
| Drabkin, D. L. | University of Pennsylvania |
| Dragstedt, Carl A. | Northwestern University |
| Dragstedt, Lester R. | University of Chicago |
| Draper, George W. | Columbia University |
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